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THE AMERICAN
JOURNAL OF PHYSIOLOGY

VOL. 46 MAY 1, 1918 No 2

THE REGULATION OF RENAL ACTIVITY

IV. Regulation of Urea Excretion by Adrenalin

T. ADDIS, G. D. BARNETT AND A. E. SHEVKY

From the Medical Division of Stanford University Medical School, San Francisco

Received for publication March 7, 1918

The general conditions we have already outlined (1) were observed. No food or water was given for about seventeen hours before the commencement of the experiment, which lasted for five hours and began about 9 a.m. During this time four collections of urine and of blood were made. No urea was given, but in order to keep the conditions uniform with those in other experiments the stomach tube was passed just before the bladder was first washed out. This procedure was carried through on a group of twenty-eight rabbits. The experiments were then repeated on the same animals under the same conditions except that 0.25 cc. of Parke, Davis & Co.'s 1-in-1000 Adrenalin Chloride was injected subcutaneously at the commencement of each of the five hours of observation. The average rates of excretion, blood urea concentrations and ratios for each of the four periods of the experiment without and with adrenalin, are given in table 1 and charted in figure 1. The details for the individual animals will be found in table 6 at the end of the paper.

The five subcutaneous injections of 0.25 cc. of 1-in-1000 adrenal solution increase the rate of urea excretion and at the same time decrease the blood urea concentration. There is, consequently, a marked increase in the ratio between the urea content of the urine and of the blood. It should be noted that the decrease in the blood urea concentration is a circumstance which in itself should have tended to lower rather than raise the ratio (2).

Reference to table 6 will show that there is a considerable degree of variation in the manner in which individual animals react to adrenalin. If these figures are compared with the control experiments without adrenalin given in the preceding paper of this series, it will be found that in some cases the increase in the ratio is very marked, while in others it is only slight. It will be noted also that there are occasional instances in which, in one period or another, the ratio is greater without than with adrenalin. It is, therefore, necessary to determine whether the actual differences noted between the average ratios without and with adrenalin might not be due to chance. If both sets of experiments were to be repeated many times a series of averages would be obtained which would differ somewhat from those we obtained. It might be found then that we had chanced to get an unusually low average with-

TABLE 1

Comparison of averages from a group of 28 rabbits without and with 0.25 cc. adrenalin

PERIOD	WITHOUT ADRENALIN			WITH 0.25 CC. ADRENALIN			ACTUAL DIFFERENCES BETWEEN RATIO AVERAGES	"PROBABLE DIFFERENCES" BETWEEN RATIO AVERAGES
	Urea in 1 hour's urine	Urea in 100 cc. of blood	Ratio:	Urea in 1 hour's urine	Urea in 100 cc. of blood	Ratio:		
	mgm.	*mgm.*		*mgm.*	*mgm.*			
I	55	68	0.71	56	52	1.04	+0.33	±0.69
II	84	67	1.17	88	51	1.86	+0.69	±0.12
III	96	71	1.27	106	52	2.21	+0.94	±0.16
IV	98	72	1.40	106	46	2.52	+1.12	±0.18

out adrenalin and an unusually high average with adrenalin, so that the difference we found was atypical and misleading. It is possible, however, to calculate from our data the "probable error" of each average and from this to obtain the "probable difference between the averages." These are given for each period in table 1. They indicate the value of the difference which would include half of all the differences between the averages without and with adrenalin which would be encountered if the experiments were repeated many times. It is clear from the fact that the probable differences are much smaller than the actual differences, that it is very unlikely that the latter are due to chance. In the case of the fourth period averages, reference to Davenport's tables (3) shows that there is only one chance in over one hundred thousand that accidental variation could bring about such a difference as actually occurred. But it is also necessary to take into considera-

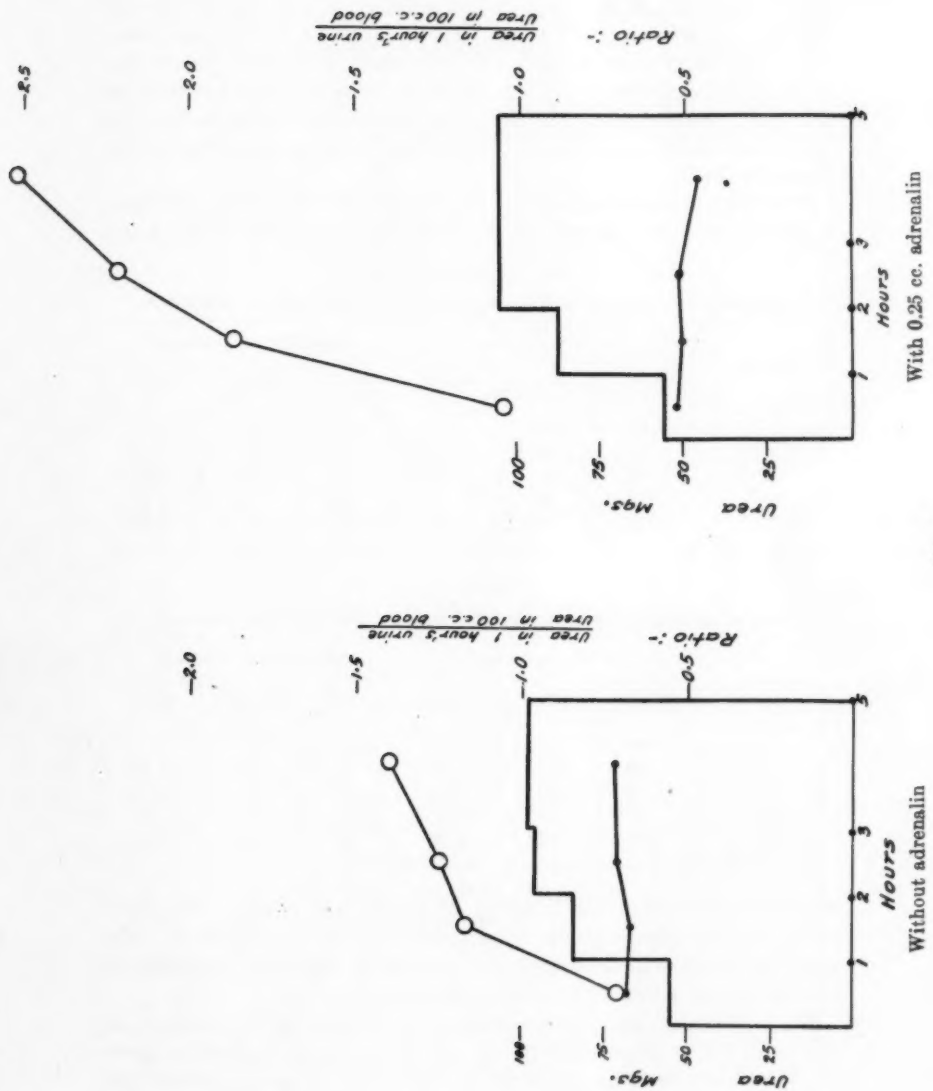
Ratio :- Urea in 1 hour's urine / Urea in 100 c.c. blood
2·5
2·0
1·5
1·0
0·5
Urea
Mgs.
100
75
50
25
1
2
3
Hours
With 0.25 cc. adrenalin
Ratio :- Urea in 1 hour's urine / Urea in 100 c.c. blood
2·0
1·5
1·0
0·5
Urea
Mgs.
75
50
25
1
2
3
Hours
Without adrenalin

Fig. 1

tion the fact that in all four periods the difference is in the direction of an increased average after adrenalin. This fact so reduces the possibility of accounting for the actual differences on the basis of chance that it becomes inappreciable. There can then be no question but that the rise in the plane of renal efficiency illustrated in the higher level of the ratio curve after adrenalin administration is a specific effect of the adrenalin itself.

The effect of adrenalin varies with the quantity injected. In figure 2 and figure 3 the averages of small groups are charted without and

TABLE 2

Comparison of a group of 6 rabbits without and with 0.125 cc. adrenalin

PERIOD	WITHOUT ADRENALIN			WITH 0.125 CC. ADRENALIN		
	Urea in 1 hour's urine	Urea in 100 cc. blood	Ratio:	Urea in 1 hour's urine	Urea in 100 cc. blood	Ratio:
	mgm.	*mgm.*		*mgm.*	*mgm.*	
I	73	89	0.71	35	71	0.48
II	96	91	1.02	50	72	0.82
III	116	86	1.19	88	71	1.26
IV	135	80	1.59	138	67	2.02

TABLE 3

Comparison of a group of 7 rabbits without and with 0.0625 cc. adrenalin

PERIOD	WITHOUT ADRENALIN			WITH 0.0625 CC. ADRENALIN		
	Urea in 1 hour's urine	Urea in 100 cc. blood	Ratio:	Urea in 1 hour's urine	Urea in 100 cc. of blood	Ratio:
	mgm.	*mgm.*		*mgm.*	*mgm.*	
I	48	61	0.74	18	42	0.42
II	74	62	1.18	35	41	0.84
III	92	64	1.45	59	44	1.40
IV	102	67	1.56	66	42	1.65

with doses of 0.125 cc. and 0.0625 cc. of 1-in-1000 adrenalin. The effect of the adrenalin does not become apparent until the fourth period. The degree of increase in the activity of the kidney, therefore, decreases as the amount of adrenalin injected is decreased.

An interesting result was obtained by increasing the amount of adrenalin. The effect of 0.5 cc. on a group of rabbits is shown in figure 4. It will be noted that the increase in the ratio is less than after 0.25 cc. The averages of three rabbits which were given 1 cc. at the

Ratio :- Urea in 1 hour's urine / Urea in 100 c.c. blood
2.5
2.0
1.5
1.0
0.5
Urea
Mgs.
25
50
75
100
1
2
3
HOURS
With 0.125 cc. adrenalin
Ratio :- Urea in 1 hour's urine / Urea in 100 c.c. blood
2.0
1.5
1.0
0.5
Urea
Mgs.
25
50
75
100
1
2
3
HOURS
Without adrenalin

Fig. 2

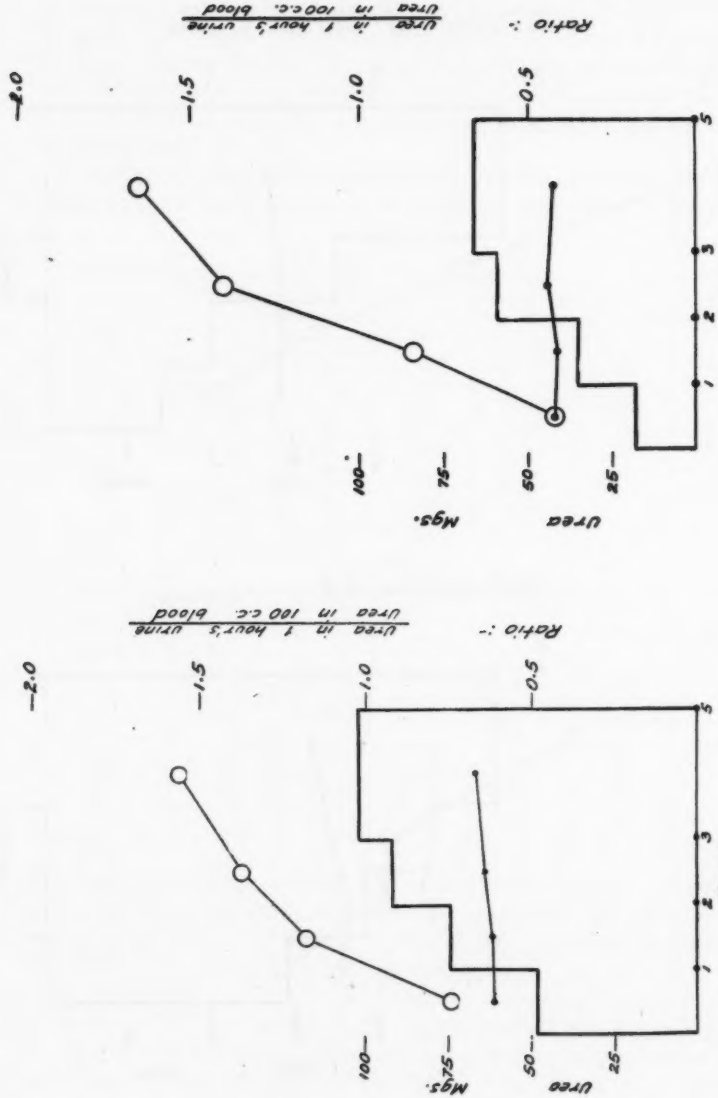
Ratio :- Urea in 1 hour's urine / Urea in 100 c.c. blood
2.0
1.5
1.0
0.5
Urea
Mgs.
100
75
50
25
1
2
3
5
Hours
With 0.0625 cc. adrenalin
Without adrenalin

Fig. 3

beginning of the first hour and 0.5 cc. at each of the four succeeding hours are charted in figure 5. Here adrenalin produces an effect which is directly contrary to that obtained with smaller doses. The decrease in the ratio is so pronounced that it would seem likely that it represents the renal reaction to the generalized toxic effect described by Elliott (4) as following large amounts of adrenalin.

TABLE 4

Comparison of averages from a group of 13 rabbits without and with 0.5 cc. of adrenalin

PERIOD	WITHOUT ADRENALIN			WITH 0.5 CC. ADRENALIN		
	Urea in 1 hour's urine	Urea in 100 cc. of blood	Ratio:	Urea in 1 hour's urine	Urea in 100 cc. blood	Ratio:
	mgm.	*mgm.*		*mgm.*	*mgm.*	
I	47	60	0.71	53	41	1.33
II	71	61	1.14	82	45	1.99
III	76	63	1.22	109	45	2.25
IV	92	68	1.41	109	47	1.96

TABLE 5

Comparison of a group of 3 rabbits without and with 0.625 cc. adrenalin (1 cc. at the commencement of period 1, and 0.5 cc. thereafter)

PERIOD	WITHOUT ADRENALIN			WITH 0.625 CC. ADRENALIN		
	Urea in 1 hour's urine	Urea in 100 cc. blood	Ratio:	Urea in 1 hour's urine	Urea in 100 cc. of blood	Ratio:
	mgm.	*mgm.*		*mgm.*	*mgm.*	
I	30	46	0.63	31	81	0.37
II	53	47	1.15	36	85	0.41
III	66	48	1.40	49	93	0.58
IV	96	50	1.93	54	95	0.60

Although the question as to the exact quantities of adrenalin effective in regulating the rate of urea excretion could be accurately decided only by slow long-continued intravenous injections, our results at least indicate that a gradation of renal stimulation is produced by increasing doses, that there is an optimal quantity which leads to a great increase in kidney action and that amounts above this optimum have less effect until, with relatively large quantities, an actual depression of kidney function results.

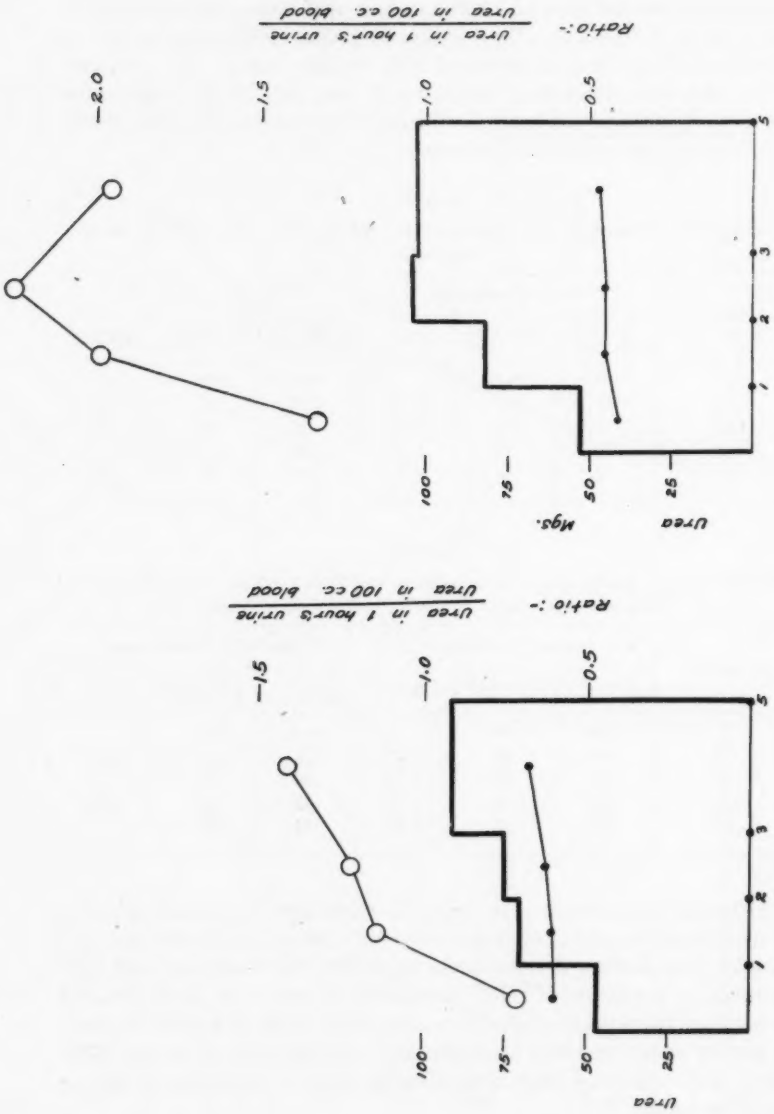
Ratio :- Urea in 1 hour's urine / Urea in 100 c.c. blood
2.0
1.5
1.0
0.5
Urea
Mgs.
100
75
50
25
Hours
1
2
3
5
With 0.5 cc. adrenalin
Ratio :- Urea in 1 hour's urine / Urea in 100 c.c. blood
1.5
1.0
0.5
Urea
100
75
50
25
Hours
1
2
3
5
Without adrenalin

Fig 4.

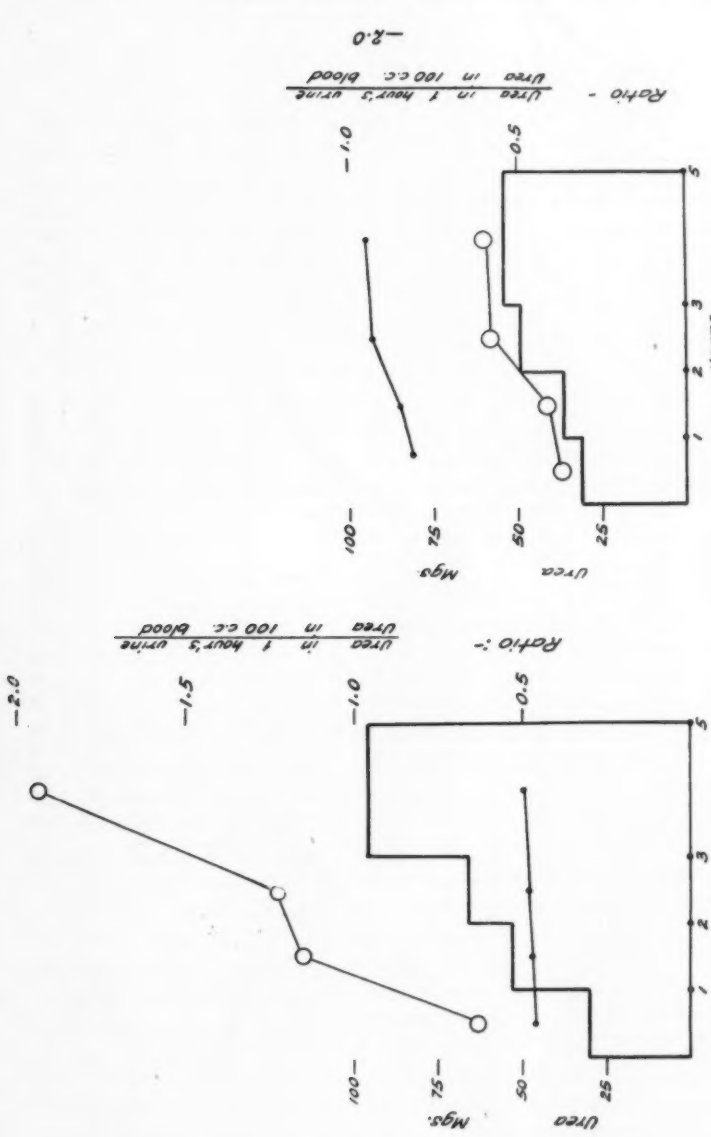
Rathio :- Urea in 1 hour's urine / Urea in 100 c.c. blood
Urea
Mgs.
25
50
75
100
0.5
1.0
1.5
2.0
1
2
3
5
Hours
Without adrenalin
Rathio - Urea in 1 hour's urine / Urea in 100 c.c. blood
With 0.6 cc. adrenalin

Fig. 5

TABLE 6

Adrenalin 0.25 cc. hourly

RABBIT NO.	PERIOD I			PERIOD II			PERIOD III			PERIOD IV		
	Urea in 1 hour's urine. Mgm.	Urea in 100 cc. blood. Mgm.	Ratio:	Urea in 1 hour's urine. Mgm.	Urea in 100 cc. blood. Mgm.	Ratio:	Urea in 1 hour's urine. Mgm.	Urea in 100 cc. blood. Mgm.	Ratio:	Urea in 1 hour's urine. Mgm.	Urea in 100 cc. blood. Mgm.	Ratio:
59	54	60	0.90	72	54	1.33	18	54	0.33	84	51	1.63
65	151	58	2.60	105	65	1.62	124	65	1.91	137	51	2.70
66	49	39	1.25	52	21	2.46	80	63	1.27	lost	lost	lost
67	32	57	0.57	104	57	1.84	133	54	2.47	130	51	2.55
71	68	45	1.52	97	30	2.95	110	51	2.15	49	51	0.96
72	lost	lost	lost	109	51	1.58	158	54	2.93	128	51	2.50
80	188	153	1.23	214	114	1.88	333	102	3.27	246	93	2.64
82	176	174	1.01	189	174	1.09	202	177	1.14	lost	lost	lost
85	28	27	1.03	46	33	1.39	54	21	2.57	78	21	3.71
86	5	30	0.15	28	30	0.95	39	21	1.86	143	23	6.20
87	45	54	0.83	126	60	2.10	210	67	3.14	120	66	1.82
88	53	69	0.77	100	69	1.45	137	64	2.14	131	63	2.08
90	46	26	1.79	93	28	3.32	86	35	2.44	114	34	3.34
93	30	45	0.68	59	48	1.23	75	48	1.56	134	45	2.98
94	49	47	1.04	75	48	1.56	70	48	1.45	65	50	1.31
95	41	51	0.80	77	53	1.45	70	66	1.07	51	69	0.74
96	23	33	0.71	40	35	1.16	53	30	1.75	34	36	0.94
97	42	45	0.93	63	33	1.91	54	44	1.22	249	42	5.94
98	43	39	1.12	26	39	0.65	73	36	2.04	90	36	2.50
99	32	40	0.81	65	42	1.51	96	33	2.91	99	35	2.83
100	29	33	0.89	81	35	2.32	49	36	1.37	40	35	1.15
102	57	45	1.27	107	44	2.44	117	45	2.60	66	48	1.36
103	38	39	0.94	109	24	4.57	124	18	6.88	124	24	5.16
104	53	56	0.95	120	63	1.90	120	68	1.77	103	69	1.50
105	30	30	1.00	47	28	1.69	43	27	1.58	47	27	1.75
68	50	36	1.38	85	45	1.90	119	44	2.70	114	45	2.53
70	16	34	0.49	51	39	1.31	70	40	1.75	82	42	1.96
73	77	52	1.47	133	54	2.46	141	39	3.62	112	42	2.68
Averages.....	56	52	1.04	88	51	1.86	106	52	2.21	106	46	2.52
Averages for the same rabbits without adrenalin.....	55	68	0.71	84	67	1.17	96	71	1.25	98	72	1.40
The amount by which ratios obtained with adrenalin exceed the average ratios without adrenalin.....			+0.33			+0.69			+0.96			+1.12

DISCUSSION

We have shown that the injection into the subcutaneous tissues of certain amounts of adrenalin is followed by a marked increase in the activity of the kidney in the excretion of urea. This fact raises the question as to whether the adrenin produced by the suprarenal glands within the body may not be one of those unknown factors in the regulation of renal function whose mode of action was discussed in the preceding paper of this series. To those who are conversant with the recent literature on adrenin this possibility may at first sight seem remote. The work, especially, of Stewart and Rogoff has failed to confirm the validity of theories under which certain physiological phenomena were related to variations in the rate of adrenin secretion. And it will be objected that we do not even know that there is any adrenin in the blood as it reaches the kidney, since sensitive involuntary muscle test-objects fail to show its presence in blood from the jugular vein (5). Nevertheless, we hold that it would be premature to assume that adrenin produced within the body may not play a part in the regulation of renal function. For apart from the possible route by which adrenin may reach the kidney directly, (6) there is, as we shall show, good reason to believe that the secretory activity of the kidney may be influenced by amounts of adrenin much smaller than those required to alter the action of involuntary muscle fibers. This question of the relation between adrenin and renal function will be more fully discussed in a later paper.

So much attention has been paid to the effect of adrenalin on muscular action that it is natural at first to suppose that its stimulating effect on the kidney is secondary to changes induced in the circulatory conditions within the organ. But the evidence as to the action of adrenalin on the renal vessels goes to show that it decreases the rate of flow of blood through the kidney. Hoskins and Gunning (7) have recently investigated the effect of the intravenous injection of both depressor and pressor doses. In every one of seventeen experiments they found the rate of flow of blood from the cut renal vein to be diminished. There would thus be an apparent contradiction in the vascular and secretory effect of adrenalin, if there were reason to believe that adrenalin given subcutaneously, as in our experiments, had any effect on the renal artery. There is, however, no such reason. When given subcutaneously the rate at which adrenalin gains entrance to the blood stream is very slow (4). In the rabbit Biedl (8) was not able to obtain a rise of blood pressure with any dose given subcutaneously. Bilberfeld (9)

found no effect on the blood pressure of rabbits after the subcutaneous injection of 2 to 5 mgm. of adrenalin. These are enormous quantities as compared with those which have a marked effect on blood pressure when given intravenously. Yet though the rate of absorption of adrenalin from the tissues is so slow that at any one time there is apparently never a sufficient concentration in the blood to produce arterial effects, we do know, from the occurrence of such a phenomenon as glycosuria, that some adrenalin reaches the blood stream. Certain adrenalin effects may thus result from quantities of a different order of magnitude from those required to produce circulatory effects. Further it must be remembered that even if it were found that a greater amount of blood passed through the kidney during the period in which a hypersecretion of urea occurs after the subcutaneous injection of adrenalin, it would still remain to be proved that the circulatory changes were the cause and not the result of the increased renal activity. For these reasons we think it highly improbable that the augmenting action of adrenalin on the secretion of urea in our experiments is the result of an adrenalin action on the blood supply of the kidney.

Does adrenalin then act as a direct stimulant to the urea secreting components of the kidney cells? All analogy is against such a view. Adrenalin, for instance, does not act on the contractile elements of muscle cells but on the myo-neural junction,—the receptive substance of Langley (10)—Elliott's generalization (4) still stands that any tissue or organ responding to adrenalin is under the control of the sympathetic nervous system, though the converse that all organs innervated from the sympathetic are sensitive to adrenalin is not invariably true. But though the kidney is richly supplied with sympathetic nerves it has not been proved that they have any direct influence on the secretory activity of the kidney, because it has always been possible to ascribe the secretory changes which follow their stimulation to the concomitant circulatory alterations which this procedure also induces. And there seems to be a tendency to accept, almost as a proved fact, the conception that the kidney is not regulated through the nervous system except in an indirect manner through its blood supply. But it is noteworthy that there is conclusive anatomical evidence which runs counter to this view. All nerve fibers do not end in the blood vessels. The glomeruli and tubules are surrounded by a quite separate and distinct plexus of nerve fibers, which end upon or between the renal cells. This was first discovered by Berkley (11) in 1893 and it has since been repeatedly confirmed, most recently by Smirnow (13) and by Renner

(14). We, therefore, believe that adrenalin influences the secretory activity of the kidney cells through the medium of something in the termination of sympathetic nerve fibers analogous to the receptive substance in the end-plates of muscle fibers.

CONCLUSION

The subcutaneous injection of adrenalin (Parke, Davis & Co.) is followed by an increase in the urea excreting activity of the rabbit's kidney. There is a certain amount of adrenalin which produces the greatest increase in function. Smaller amounts have less and less effect until there is no change from the normal. With larger amounts the augmenting effect on secretion also becomes less until, with relatively large doses, the reverse effect of a decrease in function is found. Except with these large amounts the rate of urea excretion is more rapid than in animals not given adrenalin, in spite of a lowering of the blood urea concentration.

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THE REGULATION OF RENAL ACTIVITY

V. Regulation of Urea Excretion by Pituitrin

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There is no conclusive evidence that the pituitary gland contains any substance which alters the activity of the kidney. It is true that in some pathological conditions in which the gland is involved, the volume of the urine is found to be greatly increased. It is also known that the volume of urine is increased by intravenous injection of pituitary extract, and is decreased by subcutaneous injection of the same extract. But though these facts are probably correctly explained on the assumption that some substance in the pituitary gland alters the activity of the kidney in the excretion of water, they may also be accounted for on the hypothesis that the changes in the amount of water excreted are the passive results of concomitant changes in the amount of water available for excretion. On this view the active principle in the pituitary extract might be a regulator of the amounts of water held or liberated by the tissues in general, rather than a factor controlling the capacity of the kidney cells to abstract water from the blood. A true regulation of the water excreting function of the kidneys would be proved only if it were shown that the excretion of water was changed by pituitary extract in a manner which could not be accounted for as a result of simultaneous alterations in the quantity of free water in the blood. Since there is no method by which we can measure that fraction of the total water content of the blood which is "free," the question as to which of these two explanations is correct cannot at present be decided.

But there is no difficulty of this sort in interpreting the meaning of changes in urea excretion. There is no "bound" urea in the blood. All is immediately available for excretion. And if we find that the administration of pituitary gland extract leads to any significant alteration in the rate of urea excretion which cannot be accounted for on the

basis of a change in the urea concentration of the blood, we may conclude that it is the activity of the kidney itself which has been altered.

The substance in pituitary extracts which induces the changes in the volume of urine we have referred to is present in the Pituitrin of Parke, Davis & Co. This extract also causes those changes in blood pressure and in the contractions of surviving segments of intestine which are produced by extracts of the pars intermedia of the pituitary gland.

The same methods were used as in the work on adrenalin. The pituitrin was given every hour by subcutaneous injection to a group of rabbits, and the average results compared with the averages obtained from the same group of animals under the same conditions when no pituitrin was given.

The averages from a group of nine rabbits without and with 0.25 cc. of pituitrin are given in table 1 and charted in figure 1. The details are given in table 6 at the end of the paper.

TABLE 1

Comparison of averages from a group of 9 rabbits without and with 0.25 cc. pituitrin

PERIOD	WITHOUT PITUITRIN			WITH 0.25 CC. PITUITRIN		
	Urea in 1 hour's urine	Urea in 100 cc. blood	Ratio:	Urea in 1 hour's urine	Urea in 100 cc. of blood	Ratio:
	mgm.	*mgm.*		*mgm.*	*mgm.*	
I	70	82	0.79	20	66	0.35
II	98	82	1.11	35	68	0.52
III	108	81	1.24	27	73	0.38
IV	120	85	1.44	33	77	0.43

Pituitrin decreases the rate of urea excretion although an increase in rate would be expected since the blood urea concentration is higher than in the control experiments. The combination of a low rate of excretion with a high blood concentration results in a pronounced lowering of the ratio between the urea content of the urine and of the blood.

This is exactly the reverse of the effect of adrenalin, which increases the rate of urea excretion in spite of a reduction in the blood urea concentration. It seems probable that in both cases the change in the level of the blood urea concentration is the result of the altered rate of urea excretion. Both adrenalin and pituitrin would have changed the rate to a greater extent than they actually did if it had not been for

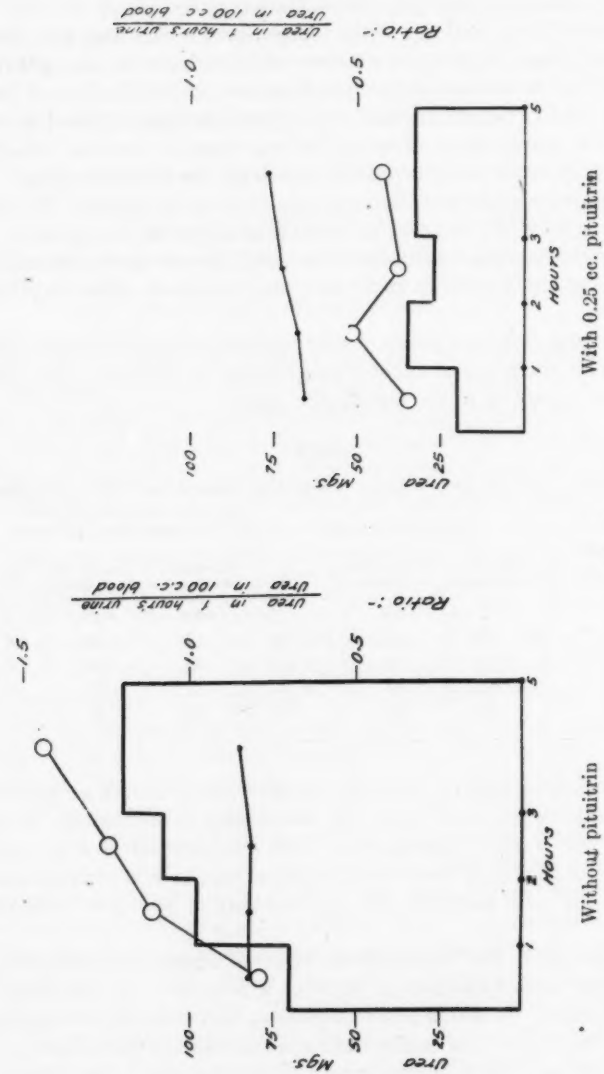

Ratio :- Urea in 1 hour's urine / Urea in 100 c.c. blood
-1.0
-0.5
Urea
Mgs.
25
50
75
100
1
2
3
5
Hours
With 0.25 cc. pituitrin
Ratio :- Urea in 1 hour's urine / Urea in 100 c.c. blood
-1.5
1.0
0.5
Urea
Mgs.
25
50
75
100
1
2
3
5
Hours
Without pituitrin

Fig. 1

this secondary effect. The hyperactive kidney lowers the blood concentration and thus automatically diminishes one important stimulus to its activity, while a kidney whose function is depressed will, by its own inaction, more and more increase a factor which tends to awake it to renewed activity. The influence of those factors other than blood urea concentration which we have grouped under the term unknown, may thus be expected to be self limited and evanescent, since a state of excess of renal activity or of inactivity carries in itself the promise of its termination. It is therefore not strange that we should have found that deviations from the average reaction to any given level of blood urea concentration are temporary phenomena observable only over short time periods.

The remaining charts (figs. 2 to 5) show the effect of decreasing amounts of pituitrin. A few experiments were also carried out with larger and smaller doses. Amounts as high as 2 cc. gave results very similar to those obtained with 0.25 cc., and quantities between 0.005 cc. and 0.00001 cc. gave no certain result. We wished to see whether with very large or very small amounts there might not be a reversal of the usual effect such as was noted with adrenalin when large doses were given, but we did not multiply such experiments and they were discontinued as soon as we were able to conclude with a reasonable degree of certainty that pituitrin in all effective doses depressed the activity of the kidney. That a certain relation exists between the degree of depression and the amount of pituitrin given, is apparent from the charts.

TABLE 2

Comparison of averages from a group of 6 rabbits without and with 0.125 cc. pituitrin

PERIOD	WITHOUT PITUITRIN			WITH 0.125 CC. PITUITRIN		
	Urea in 1 hour's urine	Urea in 100 cc. blood	Ratio:	Urea in 1 hour's urine	Urea in 100 cc. of blood	Ratio:
	mgm.	*mgm.*		*mgm.*	*mgm.*	
I	53	68	0.75	30	55	0.56
II	77	69	1.06	56	54	1.02
III	83	59	1.13	42	56	0.73
IV	106	75	1.52	46	60	0.77

TABLE 3

Comparison of a group of 6 rabbits without and with 0.0625 cc. pituitrin

PERIOD	WITHOUT PITUITRIN			WITH 0.0625 CC. PITUITRIN		
	Urea in 1 hour's urine	Urea in 100 cc. blood	Ratio:	Urea in 1 hour's urine	Urea in 100 cc. of blood	Ratio:
	mgm.	*mgm.*		*mgm.*	*mgm.*	
I	53	68	0.74	34	57	0.46
II	77	69	1.05	62	55	0.90
III	83	69	1.18	46	58	0.63
IV	98	72	1.44	27	62	0.43

TABLE 4

Comparison of averages from a group of 18 rabbits without and with 0.025 cc. pituitrin

PERIOD	WITHOUT PITUITRIN			WITH 0.025 CC. PITUITRIN		
	Urea in 1 hour's urine	Urea in 100 cc. blood	Ratio:	Urea in 1 hour's urine	Urea in 100 cc. of blood	Ratio:
	mgm.	*mgm.*		*mgm.*	*mgm.*	
I	50	62	0.75	44	74	0.76
II	69	58	1.16	67	69	1.05
III	83	65	1.24	61	73	1.00
IV	92	64	1.46	73	77	1.00

TABLE 5

Comparison of averages from a group of 4 rabbits without and with 0.0125 cc. pituitrin

PERIOD	WITHOUT PITUITRIN			WITH 0.0125 CC. PITUITRIN		
	Urea in 1 hour's urine	Urea in 100 cc. blood	Ratio:	Urea in 1 hour's urine	Urea in 100 cc. of blood	Ratio:
	mgm.	*mgm.*		*mgm.*	*mgm.*	
I	61	73	0.83	31	90	0.41
II	90	74	1.19	45	94	0.53
III	96	74	1.28	51	95	0.54
IV	113	77	1.48	76	91	0.90

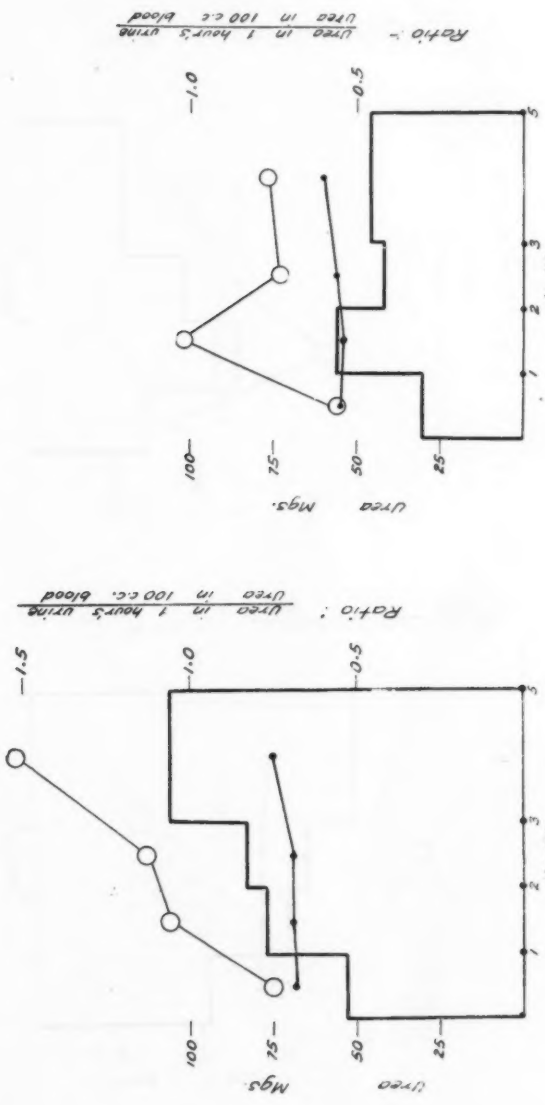
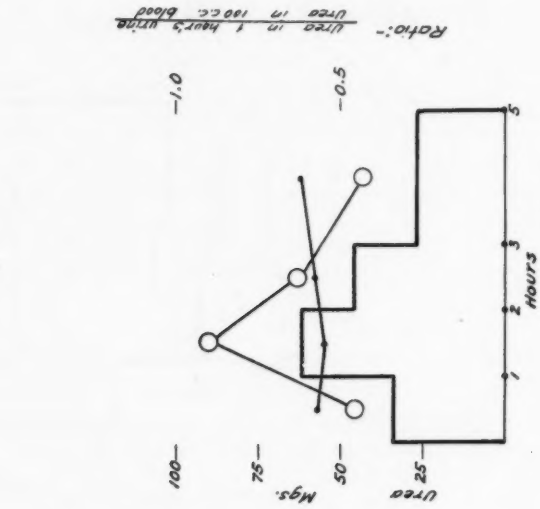
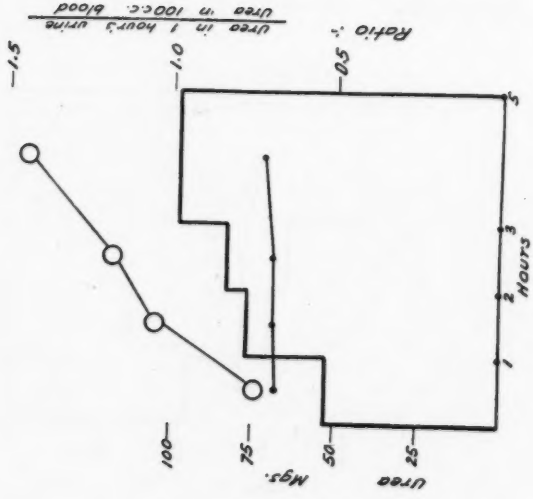

Ratio : Urea in 1 hour's urine / Urea in 100 c.c. blood
Urea Mgs.
100
75
50
25
1.0
0.5
1
2
3
HOURS
With 0.125 cc. pituitrin
Ratio : Urea in 1 hour's urine / Urea in 100 c.c. blood
Urea Mgs.
2.0
1.5
1.0
0.5
100
75
50
25
1
2
3
HOURS
Without pituitrin

Fig. 2



With 0.625 cc. pituitrin.



Without pituitrin

Fig. 3

Ratio :- Urea in 1 hour's urine / Urea in 100 c.c. blood
1.0
0.5
100
75
50
25
Mgs. Urea
1
2
3
5
Hours
With 0.025 cc. pituitrin
Ratio : Urea in 1 hour's urine / Urea in 100 c.c. blood
1.5
1.0
0.5
100
75
50
25
Mgs. Urea
1
2
3
5
Hours
Without pituitrin

Fig. 4

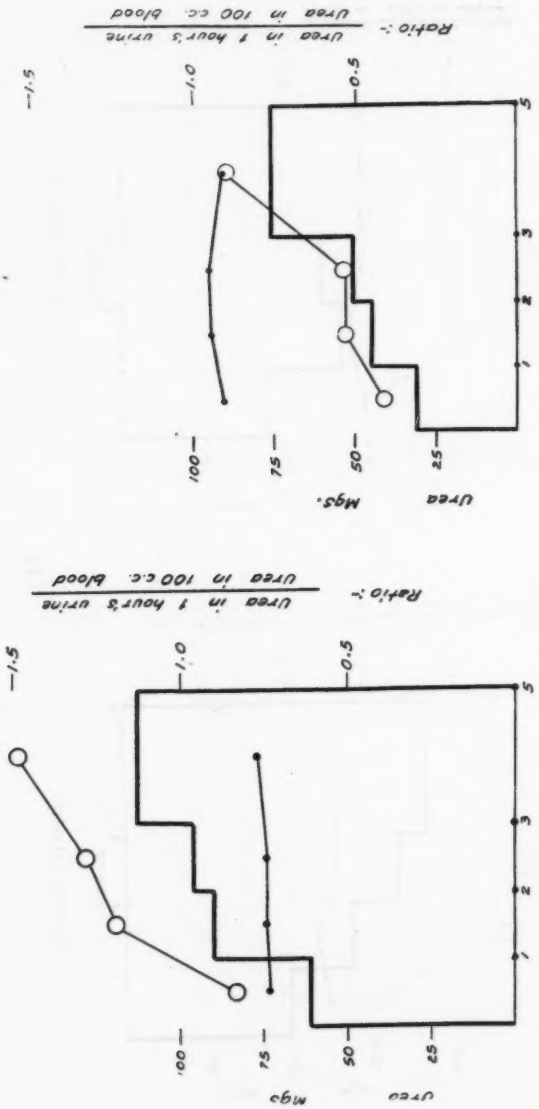

Ratio :- Urea in 1 hour's urine / Urea in 100 c.c. blood
1.5
1.0
0.5
Urea
Mgs.
100
75
50
25
1
2
3
5
Hours
With 0.0125 cc. pituitrin
Ratio :- Urea in 1 hour's urine / Urea in 100 c.c. blood
1.5
1.0
0.5
Urea
Mgs.
100
75
50
25
1
2
3
5
Hours
Without pituitrin

Fig. 5

TABLE 6

Pituitrin 0.25 cc. hourly

RABBIT NO.	PERIOD I			PERIOD II			PERIOD III			PERIOD IV		
	Urea in 1 hour's urine. Mgm.	Urea in 100 cc. blood. Mgm.	Ratio:	Urea in 1 hour's urine. Mgm.	Urea in 100 cc. blood. Mgm.	Ratio:	Urea in 1 hour's urine. Mgm.	Urea in 100 cc. blood. Mgm.	Ratio:	Urea in 1 hour's urine Mgm.	Urea in 100 cc. blood. Mgm.	Ratio:
59	0	46	0.00	18	60	0.29	23	76	0.30	24	63	0.38
65	30	38	0.81	30	37	0.80	34	36	0.95	28	36	0.76
66	12	93	0.13	17	66	0.25	17	59	0.24	31	84	0.38
67	8	61	0.13	19	63	0.30	5	64	0.08	2	64	0.05
71	15	75	0.20	0	75	0.00	2	84	0.03	16	93	0.17
72	33	60	0.55	60	90	0.66	55	96	0.58	90	105	0.86
85	50	55	0.91	59	60	0.98	7	66	0.11	21	75	0.28
86	22	79	0.27	31	81	0.38	43	83	0.52	28	89	0.31
88	11	85	0.13	81	82	0.99	54	84	0.64	59	86	0.68
Averages	20	66	0.35	35	68	0.52	27	73	0.38	33	77	0.43
Averages obtained from the same rabbits without pituitrin	70	82	0.79	98	82	1.11	108	81	1.24	120	85	1.44
The amount by which the average ratios obtained after pituitrin are less than the average ratios obtained without pituitrin			−0.44			−0.59			−0.86			−1.01

DISCUSSION

We have shown that the subcutaneous injection of pituitrin is followed by a marked depression of the activity of the kidney in the excretion of urea.

There is no more ground for the supposition that this depressing action of pituitrin is the result of circulatory changes in the kidney than there is for ascribing the accelerating action of adrenalin to alterations in renal blood supply. What we know of the effect of these substances on the vessels of the kidney stands in direct opposition to any such hypothesis. Pituitrin given intravenously increases the volume of the kidney and causes diuresis (1). Adrenalin given intravenously de-

creases the volume of the kidney and stops the flow of urine (1). Outside the body pituitrin dilates the renal artery while adrenalin constricts it (2). If they have any such influence on the renal vessels when given subcutaneously one would expect the circumstances to counteract, rather than to cause the changes in kidney activity which we have shown they produce. But as a matter of fact there is no reason to suppose that pituitrin absorbed from the tissues has any vascular effects at all. Taukow (3) found that in rabbits, the subcutaneous injection of as much as 5 cc. of the extract we used did not alter the blood pressure. Yet one-hundredth part of this amount given in the same way will markedly reduce the urea excreting activity of the rabbit's kidneys. Here, as in the case of adrenalin, there seems reason to distinguish between the effect on the action of involuntary muscle and on the secretory action of the kidney, in the sense that very much smaller concentrations are more effective in the one case than in the other.

CONCLUSION

The subcutaneous injection of pituitrin (Parke, Davis & Co.) is followed in all effective amounts by a decrease in the urea excreting activity of the rabbit's kidney. The rate of urea excretion is slower than in animals not given pituitrin, although the blood urea concentration is higher.

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THE ACTIVATION OF MUSCLE CATALASE BY LIVER

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It is well known that thyroid feeding increases oxidations, and the results herein described are a sort of by-product of an attempt to ascertain if the thyroid could in any way be connected with catalase. Rat carcinoma was chosen for the reason that it is rich in catalase, and it was thought that there was a possibility of showing some connection between the thyroid and catalase that might account for the oxidation processes that must be going on where there is such a rapid cell division. In seeking for a control it was observed that the addition of small quantities of liver very markedly increased the activity of the catalase. The original problem was therefore dropped for the time being, and attention given to this phenomenon.

The liver and the leg muscles of the rat were used, as being at the two ends of the scale of catalytic activity; the liver being most active and the muscle least so, if we except the brain. The animals were killed and immediately perfused with a solution of sodium chloride of m/6 concentration and made up with tap water. A cannula was introduced into the aorta and the solution allowed to run until the fluid coming from the jugular vein was clear. Incisions into the liver showed it to be free from blood. The liver and muscles were then removed, passed through a meat grinder and the pulpy mass thus obtained was used in weighed quantities.

Commercial hydrogen peroxide was diluted with an equal volume of distilled water, making a 1.5 per cent solution of H_2O_2. It was used both neutralized and unneutralized. The oxygen was collected in a burette over water in the usual way, the amount given off in ten minutes being taken as the standard. As some of the work was done at noon and some at night, there was a variation of several degrees in the temperature and therefore the gas volumes are all reduced to 0° and 760 mm. Hg, the fractions being disregarded. Unless otherwise stated, 50 cc. of the peroxide solution were used.

The acceleration of the catalase activity of muscle by adding a small amount of liver is shown by the following:

1 gram of muscle liberated 24 cc. of oxygen.
1 gram of liver liberated 156 cc. of oxygen.
0.9 gram of muscle + 0.1 gram of liver, gave 126 cc. of oxygen.
0.98 gram of muscle + 0.02 gram of liver, gave 58 cc. of oxygen.

The objection might be made that as the liver contains so much more catalase, we are dealing here simply with an increase of the active mass; 0.98 gram of muscle plus 0.02 gram of liver represents about 1.05 grams of muscle. This slight increase in amount could hardly cause an increase of 140 per cent in activity.

To make certain of this point, the following combination was made:

1 gram of muscle gave 50 cc. of oxygen.
1 gram of liver gave 244 cc. of oxygen.
0.5 gram of muscle + 0.1 gram of liver gave 250 cc. of oxygen.

Assuming the amount of catalase in the liver to be five times greater than in muscle, the above combination of muscle and liver represents 1 gram muscle; yet the amount of oxygen given off is that of the liver rather than of the muscle.

0.5 gram of muscle gave 23 cc. of oxygen.
0.1 gram of liver gave 41 cc. of oxygen.

The sum of these, 64 cc., is far below that for the same quantities when used in combination.

The following was then tried, assuming the liver to contain ten times as much catalase as the muscle:

0.5 gram of muscle + 0.02 gram of liver gave 210 cc. of oxygen.

In the earlier experiments other tissues of the same animal were used as controls. For instance, in experiment 7, rat,

1 gram of muscle gave 38 cc. of oxygen.
1 gram of liver gave 203 cc. of oxygen.
0.9 gram of muscle + 0.1 of liver gave 200 cc. of oxygen.
0.9 gram of muscle + 0.1 gram of blood clot gave 158 cc. of oxygen.
0.9 gram of muscle + 0.1 gram of thyroid gave 44 cc. of oxygen.
0.9 gram of muscle + 0.1 gram of spleen gave 51 cc. of oxygen.
0.9 gram of muscle + 0.1 gram of kidney gave 84 cc. of oxygen.
0.9 gram of muscle + 0.1 gram of pancreas gave 32 cc. of oxygen.
0.9 gram of muscle + 0.1 gram of testes gave 29 cc. of oxygen.

It will be seen that there is a small amount of acceleration with all but the pancreas and testes, where there seems to be a slight retardation. It should be pointed out that in the case of the spleen and kidney, they were not entirely free from blood. It is rather difficult, in perfusing the entire animal, to rid these organs of all blood. Aside from this it is quite evident that the liver has an accelerating effect far in excess of the other organs, and that the blood comes next in efficiency.

These experiments were done with acid H_2O_2. They were repeated with freshly neutralized H_2O_2 with the same results, the difference being in the magnitude of the figures. It was noticed, however, that whereas the liver acted much more energetically in the neutral peroxide, the muscle did not seem to be affected by the reaction; it acted as well in the acid as in the neutral peroxide.

These are the facts. The interpretation of these facts is not so clear. Some years ago Battelli and Stern (1) claimed to have obtained a substance, philocatalase, which had the property not only of antagonizing anticatalase but also of regenerating the catalase. They also describe an "activator" of the philocatalase. DeWaele and Vandevelde (2) throw doubt upon the existence of anticatalase, and therefore upon the existence of philocatalase and its activator. Loevenhart (3) observed an acceleration when pancreas and liver, and muscle and liver were combined, but he regarded it as an activation of the liver by the pancreas and muscle and thinks it due to a neutralization of the acid by some substance contained in these tissues. It has already been stated that in the present observations it was noted that liver was retarded by the acid, and that muscle acted equally well in acid or neutral peroxide, but when they were added together there was a more marked acceleration in the neutral peroxide. As an example,

0.5 gram of muscle + 0.02 gram of liver, acid H_2O_2, gave 170 cc. oxygen.
0.5 gram of muscle + 0.02 gram of liver, neutral H_2O_2 gave 240 cc. oxygen.

From these results it is clear that there is something in the liver catalase that does not exist in the muscle, or that liver catalase is different from muscle catalase.

In a preliminary communication read before the Pacific Coast Branch of the Society for Experimental Biology and Medicine at its January meeting, it was suggested tentatively that the liver secreted an activator of catalase, possibly in the nature of an internal secretion, and it might be well to point out the reasons for such a suggestion. In the first place, the blood is nearly as effective as the liver, and this one would

expect if we are dealing with an internal secretion. Also the slight accelerating effect of practically all the organs could thus be accounted for, as well as the varying catalytic activity of the different tissues. Burge (4) describes an increase of catalase in muscle during activity, and this might be due as well to an increased amount of an accelerator carried to the muscle by the increased blood supply, as to an actual increase in the amount of catalase. So far as I know his estimations were all based upon the catalase activity of the muscle and not on the actual determination of the amount of catalase present. Finally, experiments now in progress with the catalase of liver obtained by the method of Battelli and Stern (5), seem to show that the accelerating property disappears in the preparation of the catalase. Enough work along this line has not been done, however, and it must be left for a future communication if the times, so sadly out of joint, will permit.

SUMMARY

1. In both acid and neutral hydrogen peroxide, the addition of a small amount of liver to muscle increases the catalytic activity of the mass.

2. Blood also has an accelerating effect on muscle catalase, nearly equal to that of liver.

3. It is suggested tentatively that this accelerating action may be due to an internal secretion, and the reasons for such a suggestion are given.

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MUSCULAR STRENGTH AND MUSCULAR SYMMETRY IN HUMAN BEINGS

I. In Children

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The observations on which this report is based were obtained in connection with a study of the causes and treatment of infantile paralysis, conducted under the auspices of the Vermont State Board of Health and financed through the generosity of an anonymous donor.

The series of papers of which this is the first represents an attempt to elucidate the factors which determine effective muscular strength in human beings, as displayed in maximal volitional efforts. The application by Weber and others (1) of the principle of absolute muscular force to human muscles gives us some idea of the relative strength of isolated human muscles in comparison with similar muscles of lower animals; the numerous investigations with the ergograph (2) throw much light on the conditions which influence muscular endurance; the literature of physical training contains many data relative to "strength tests" applied in gymnasia to athletes, and in a few cases for the purpose of aiding in the prescription of therapeutic exercise (3); but none of these has given rise to a generalization which will enable us to forecast for any individual his probable maximum strength and to analyze observed departures from expectation. Aside from the familiar fact that strength increases with exercise we have had hitherto practically no data on which to base such a generalization.

Effective muscular strength, by which is meant the power developed at the actual points where strength is ordinarily exerted, depends on a number of factors. The muscles work for the most part in groups, and through action on levers. The effective strength, for example, of the calf muscles in such an action as rising on the toes has a complex mechanical basis, in comparison with the absolute muscular force of the isolated gastrocnemius. Moreover, in exhibitions of voluntary

muscle power the mechanism involved is a neuro-muscular one, in which the nervous part may have as much hand in determining the degree of activity as the purely muscular. Thus, so far as the actual use of the muscles is concerned, effective strength rather than intrinsic muscle power is of significance.

Source of the material. In connection with the development and use of a system of muscle-testing intended primarily as a feature of the after-care of infantile paralysis (4), a large series of observations of normal muscular strength was obtained, Part of the tests were made in the Orthopedic Department of the Children's Hospital, Boston. Most of the others were made at various points in Vermont.

The muscle test. Detailed descriptions of the system of muscle-testing are given elsewhere (4), (5). For the present purpose only the general features of the test will be described. The value obtained for each muscle-group is the "breaking strength." By this is meant the tension shown on a spring-balance at the instant the resistance of the contracted muscle is overcome by a pull in the opposite direction, exerted through the balance. Traction is afforded by a sling placed at a selected point on the part in which the muscle-group has its insertion. Standard positions for the sling and for the regions involved are used. These are so selected as to be easily located and to afford satisfactory mechanical conditions. Care is taken throughout that all purely physical factors, such as line of pull, shall be kept as nearly constant as possible. In this investigation the readings were taken in pounds and the attempt was made to read to the nearest half-pound. Tests on eleven muscle-groups on each arm and ten on each leg were used, a total of forty-two. These are enumerated in table 2. An additional arm group, the abductor of the thumb, was tested, but this is so weak, especially in young children, that the probable error on a scale read only to half-pounds is too great to justify its inclusion in a statistical study. This group is omitted, therefore, in all the data considered herewith.

Subjects. Tests available for this study were had on two hundred and forty individuals. These included one hundred and twenty-eight males and one hundred and twelve females. All ages were represented from four to eighteen. The age distribution is given in table 1. Since only the most advanced four-year old children could be reliably tested they are not listed separately, but are included under age 5. Many of the subjects (one hundred and sixty-eight) were patients on whom the diagnosis of infantile paralysis had been made. In all save

seven the acute stage was a year or more in the past. The others were such as came to hand conveniently while tests were in progress. Many of them had localized abnormalities, such as club-foot or slight scoliosis, but in no case were the abnormalities of such a character as to vitiate the value of the readings taken. The group of subjects as a whole was fairly representative. There were a few cases from the slums of the city in which there was obvious under-nourishment. In most of the cases, however, there was no indication of malnutrition. A large percentage of the subjects were cripples in whom active exercise was a

TABLE 1

Subjects tested, grouped according to sex and age

AGE	MALES	FEMALES	TOTAL
5	16	12	28
6	11	8	19
7	20	17	37
8	16	16	32
9	14	17	31
10	6	11	17
11	13	4	17
12	6	4	10
13	3	6	9
14	1	2	3
15	6	1	7
16	6	5	11
17	6	2	8
18	4	7	11
Totals.....	128	112	240

matter of difficulty. Although one would prefer for a study of this sort subjects who had not suffered from a disabling illness, the opportunity of obtaining a series of normal children even approximately as extensive as this seemed too remote to justify postponement of the present analysis. It may be said, furthermore, in favor of this group of subjects, that the less amount of activity would tend to diminish the occurrence of special exercise effects, leaving the group particularly representative of the conditions determining general muscular strength, independent of the effects of specific exercises. In this respect it was, perhaps, as satisfactory as would have been a group of strictly normal individuals.

Musuclar symmetry. The expression of strength in this study is taken as the sum of the observed strengths of the muscle-groups listed in table 2. Inasmuch as so large a proportion of the subjects had some muscle-groups not of normal strength, and therefore not possible of inclusion, a method had to be devised at the outset by which allowance could be made for the omitted muscle-groups in stating the total strength of the individual. The method adopted was to determine for this series of subjects the average percentage distribution of strength among the various muscle-groups. As a first step in this procedure the subjects were classified by ages. Then the average strength of each muscle-group was determined for all the subjects of a given age. On the basis of these average figures the percentage distribution of the total strength among all the muscle-groups was determined for each age and the result tabulated. An interesting point brought out by study of this tabulation is confirmation, for children, of the fact established by Kellogg for adults (loc. cit., table 1) that there is much less difference between the right and left sides of the body than is generally supposed. In fact, the percentage differences between the two sides are neither great enough nor constant enough to involve serious error if the two sides of the body are assumed to be equally strong. In order to simplify the mathematical procedure this assumption was adopted. It was found, furthermore, that the percentage distributions of muscular strength for the various ages fall naturally into three series. The first of these includes the ages 5, 6 and 7; the second the ages from 8 to 12 inclusive; and the third the ages from 13 to 18 inclusive. For the practical purposes of this study the averages for the different muscle-groups for these age groupings were taken. These are set forth in table 2. In a later section, under the heading "Strength Distribution" the validity of table 2 is considered statistically. As a matter of interest, the actual average percentage distribution of strength for the two sides of the body and for the arms and legs, for the different age groups, is given in table 3.

The calculation of total strength.—The method of computing total strength when some muscle-groups are missing is as follows: The aggregate strength of the muscle-groups on which tests have been made is determined (designated A); from the figures of table 2 the percentage of the total strength represented by those muscle-groups is found (designated P); the calculation of the theoretical entire strength (designated T) is then made according to the formula $T = \frac{A}{P}$. As a check upon

TABLE 2

Average percentage distribution of strength among the muscles

MUSCLE-GROUP	AGE		
	5 to 7	8 to 12	13 to 18
Feet			
Plantar flexion	7.60	9.30	9.30
Dorsal flexion	3.35	3.10	3.20
Inversion	2.05	1.95	2.10
Eversion	1.95	1.85	2.00
Hips			
Adduction	1.60	1.50	1.55
Abduction	1.50	1.45	1.50
Extension	3.15	3.05	3.00
Flexion	3.35	3.20	3.10
Knees			
Extension	3.50	3.20	3.30
Flexion	1.80	1.70	1.70
Shoulders			
Pectoralis	2.20	2.10	2.10
Latissimus dorsi	1.45	1.50	1.45
Anterior deltoid	2.00	2.00	2.00
Posterior deltoid	1.45	1.45	1.50
Forearms			
Extension	1.95	1.75	1.60
Flexion	2.60	2.55	2.50
Wrists			
Extension	1.25	1.25	1.35
Flexion	2.25	2.15	1.90
Fingers			
Extension	0.70	0.70	0.70
Flexion	2.75	2.75	2.75
Thumbs			
Adduction	1.55	1.50	1.40

TABLE 3

Actual percentage distribution of strength

REGION	AGE		
	5 to 7	8 to 12	13 to 18
Left side	49.40	49.30	49.70
Right side	50.60	50.70	50.30
Arms	40.40	39.30	38.80
Legs	59.60	60.70	61.20

this method of computing total strength and also in an attempt to establish a means of obtaining an approximate measure of strength without actually having to carry through complete tests, the same method of calculation was applied, using only the extensors and flexors of the forearm and the extensors and flexors of the wrist as the basis for calculation. It appears that in a large proportion of individuals the strength calculations based only on the muscles of the forearm and wrist agree reasonably with the results of the complete tests. In the one hundred and twenty-six cases of this series in which this comparison was made, all ages and both sexes being represented, one hundred and seven (85 per cent) agree within 15 per cent, and one hundred and eighteen (94 per cent) agree within 20 per cent. The Pearson coefficient of correlation for this comparison is 0.91 ± 0.0168. It seems fair to conclude that strengths calculated from tests that are nearly complete are valid within the limits imposed by the inherent errors of the test itself.

After the strengths of all the individuals in each age group had been calculated averages were made for each sex separately at each age from 5 to 18 years. These averages are set down in table 4. As is at once apparent from scrutiny of the table, the values here given are not to be looked upon as satisfactory final estimates of the average strengths for the various ages of human beings. The number of cases in this series is too small, particularly at some ages, to afford a basis for such final estimates. As a preliminary toward the establishment of definite figures for the mean strength at each age they serve a purpose, however.

The relation of strength to weight. The next step was to determine whether these figures of average strength could be related with any other known data which vary with age. The obvious comparison is with weight, which varies with age in a definite manner and in which, moreover, the variations have been worked out carefully by Bowditch (6) and others. In table 4 the average strengths for the various ages are tabulated opposite the average weights of the same subjects. That there is a definite relationship between strength and weight is at once apparent when the columns in the table headed $\frac{Strength}{weight}$ are examined. The figures in these columns, when taken column by column, so as to divide the sexes, show an approximate constancy that is too striking to be accidental and that indicate a definite relationship between strength and weight. The column for the ratio of strength to weight in males shows considerable divergence at the two ends. If the figures for ages 5, 17 and 18 are omitted the average of the entire column is

20, the mean divergence from this average being slightly less than 4 per cent, and the maximum divergence 10 per cent. The figure 20 seems thus to represent fairly the ideal ratio of strength to weight in male children. The average for the column of ratios of strength to weight in females, omitting ages 15 and 17, at which there were insufficient data, is 18, with a mean divergence of 6.8 per cent, and a maximum divergence of 19 per cent. The figure 18 may be looked upon as the

TABLE 4

Ratio strength to weight; both sexes; ages 5 to 18 years

AGE	MALES			FEMALES			AVERAGE HEIGHT, BOTH SEXES
	Average weight	Average strength	Strength / weight	Average weight	Average strength	Strength / weight	
	lbs.	*lbs.*		*lbs.*	*lbs.*		*inches*
5	37	645	17.4	37.0	650	17.3	41
6	41	830	20.2	42.0	700	16.7	44
7	49	970	19.8	46.0	875	19.0	47
8	53	1050	19.8	58.0	1020	17.6	49
9	59	1260	21.0	55.0	1040	18.9	51
10	68	1380	20.2	62.5	1140	18.6	53
11	72	1490	20.7	79.5	1265	15.9	56
12	85	1600	18.8	104.0	1610	15.1	58
13	88	1750	19.9	92.0	1640	17.9	60
14	127	2300*	18.1	94.0	1710	18.2	62
15	102	1870	18.3	135.0	1760*	13.1	64
16	113	2380	21.1	114.0	2450	21.5	66
17	113	2680	23.2	83.5	1220†	14.6	67
18	155	3590	23.2	135.0	2500	19.1	67

* One case only.

† Two cases only, both severely affected by previous attacks of infantile paralysis.

ideal ratio of strength to weight in female children. The coefficient of correlation between strength and weight is, for male children, 0.93 ± 0.009, and for female children, 0.86 ± 0.019.

The ratio for male children at age 5 is seen in the table to be 17.4. This figure is so closely approximate to the general ratio for female children as to suggest that at that age there is no sex difference in respect to strength. A point that may be worth making in this connection is that in this series of subjects the average weights at age 5 are the same for both males and females (see table 4). If one takes the ground that both weight and strength are infantile in type up to this age the

suggestion follows that the female standard represents a continuation of the infantile ratio, while in the male there is a departure therefrom in the direction of a greater strength per unit of weight. It should be noted that the difference of ratio in favor of the male is not great, being only 10 per cent. The ratios for ages 17 and 18 in males are definitely higher than the average for the earlier ages. These higher ratios indicate that in adults there may be regularly higher ratios than in children, and that boys of 17 and 18 are attaining the adult condition in this regard.

TABLE 5

Comparison of actual strengths for the various ages with theoretical strengths computed by multiplying the Bowditch figures for weight by the ratios of strength to weight as established for the two sexes

AGE	MALES			FEMALES		
	Bowditch weight	Calculated strength	Actual strength	Bowditch weight	Calculated strength	Actual strength
5	41.1	740	645	39.7	710	650
6	45.2	905	830	43.3	780	700
7	49.1	980	970	47.5	855	875
8	53.9	1080	1050	52.0	935	1020
9	59.2	1180	1260	57.0	1030	1040
10	65.3	1310	1380	62.3	1120	1140
11	70.2	1400	1490	68.8	1240	1265
12	76.9	1540	1600	78.3	1410	1610
13	84.8	1700	1750	88.6	1600	1640
14	94.9	1900	2300*	98.4	1770	1710
15	107.1	2070	1870	106.1	1910	1760*
16	121.0	2420	2380	112.0	2020	2450
17				115.0	2070	1220†
18				115.2	2075	2500‡

* One case only.

† Two cases, both severely paralyzed.

‡ The average weight of these subjects was 135 lbs.; 20 lbs. more than the Bowditch figure.

As a further check upon the validity of the ratios of strength to weight here proposed, the theoretical strengths for each age in males and females were calculated by multiplying the mean weights given in the Bowditch table by the proposed ratios namely, 20 for males from 6 to 16 years of age and 18 for males of 5 years and all females. These theoretical strengths (see table 5) were then compared with the actual

strengths previously found. If we omit from the comparison age 14 in males and ages 15, 17 and 18 in females, the first three because of insufficient data and the last because the 18 year old females of this series greatly outweighed the Bowditch figure for this age, the mean difference between the theoretical and the actual strength-averages is only 6.1 per cent. The coefficient of correlation between the two sets of averages is 0.977 ± 0.007. If all ages are included except age 17 in females, which obviously should under no conditions be counted, the mean difference becomes 7.1 per cent. This very close correspondence strengthens the view that in the ratios proposed we have a close approximation to what we may call the normal relationship between the strength of the selected series of muscle-groups on which this study is based and the body-weight.

The strength-weight ratios proposed above were determined on the basis of average strengths and average weights for each age. If they represent truly the relationship assumed for them they should apply to individuals as well as to averages, and they should hold good in those subjects whose weight does not correspond with the average for their age as well as in those whose weight agrees with age expectation. To test their individual application in these regards is the purpose of the next portion of this paper. Among the subjects tested were ninety-eight males and ninety-one females, one hundred and eighty-nine altogether, whose weights were determined at the time the tests were made. Unfortunately not all the cases examined could be weighed because in several of the towns in which tests were carried on it was not found possible to obtain the use of suitable scales. The average weights set down in table 4 were calculated from the subjects that were actually weighed, and the average strengths were calculated from all the subjects of the proper age, regardless of whether their weights were known or not. Thus the two sets of averages are not based on identical cases throughout. This makes it all the more desirable to check the assumed strength-weight ratios by applying them to individuals. A simple means of doing this is to determine the strength-weight ratio for each subject whose weight is known and then to find the average for each sex separately. When this was done the average for males was found to be 19.9 and for females 18. These ratios agree closely enough with those obtained above to serve as verification of them.

A well-known element in the acceptance of averages as truly representative is that the individual data on which they are based shall be shown to cluster around them within reasonable limits. It is not easy

to decide for this set of data what are the proper limits; in other words, to tell how widely the strength of an individual may vary from the theoretical and he still be considered of "normal" strength. In view of the many factors that may influence strength and of the errors that are bound to inhere in a system of tests based on volition, it seems to me reasonable to allow a variation of 15 per cent on either side of the theoretical before considering the subject to be either below or above "normal." Of the one hundred and eighty-nine cases here under consideration one hundred and nineteen (63 per cent) were of "normal" strength according to the criterion just proposed. Thirty-five (18.5 per cent) were below "normal" and an equal number above "normal." Since the limits of "normality" are purely empirical, as they must be on so meager data, caution must be observed in drawing conclusions as to the factors which condition departures from "normality." It happens that in this series the individuals listed as "not normal" are equally divided between the sexes. This is true both of those that are of less than "normal" strength and of those that are stronger than the average. There is nothing to indicate a greater liability to divergence at some ages than at others although this series is not large enough to bring out such a tendency even though one were present. Scrutiny of the individual cases included under the captions "less than normal" on the one hand and "more than normal" on the other suggests that many of them were actually weaker or stronger than the average for their ages, so that their presence in the series helps to account for the relatively large proportion of the total which falls outside the "normal" limits. Thus half of those in the list of "weaker than normal" were infantile paralysis cases in which the test of strength was made approximately a year after the onset of the disease. The percentage of similarly recent onsets among the "normally strong" cases is less than half as large. Almost without exception the children in this group presented an appearance of delicacy which would naturally be associated in the mind of an observer with less than normal muscular strength. The group of "more than average strength" consisted of children who were, to the most casual observation, of unusual ruggedness and vigor. More than half of them, nineteen out of thirty-five, were undersized. That is, they were both shorter and lighter in weight than the average for their ages. One would be inclined to expect to find a tendency toward a high strength-weight ratio among those who are small for their ages, provided the small size does not involve a serious deficiency in amount of muscular tissue. On the whole, the

departures from "normality" in this series do not seem to be more extensive than may properly be anticipated. The further condition to be fulfilled by the strength-weight ratios, as stated above, is that they shall hold for individuals whose weight falls outside the expectation for their age as well as for those whose weight accords with age-expectation. Among the one hundred and eighty-nine cases making up the series now under examination there were seventy-seven whose weight varied from the average for their age by five pounds or more. Forty-five of these were underweight and thirty-two overweight. Twenty-three of the overweight (72 per cent) were included among the "normally strong." Seven were weaker than "normal" and two stronger Twenty-one of the underweight, slightly less than half, were of "normal" strength. Nineteen, as stated above, were stronger than "normal" and five weaker. Except for the probability, already mentioned, that undersized persons are likely to have a high strength-weight ratio, it is seen, thus, that the adopted ratios hold for individuals who are not of average weight for their age.

The relation of strength to height. Another factor which varies in a fixed manner from year to year of age and which, therefore, may properly be compared with strength, is height. At first glance one would be inclined to suppose that effective strength would vary inversely with height so long as other factors remained constant. The greater length of arms and legs would appear to diminish the effective leverage of many muscle-groups. As a matter of fact this series of subjects gave no indication that undue height is accompanied by relatively less strength. Of twenty-three individuals who were decidedly taller than the average for their weight only two showed less than "normal" strength. Five, on the other hand, were stronger than "normal." In general the relationship of strength to height seems to be that which should follow from the ratio of strength to weight as stated above and the relationship of height to weight as determined by the principle that the mass varies as the cube of the length. Since this principle applies in human beings to the ratio of weight to height and since the relationship of strength to weight is direct, it follows that the strength should vary as the cube of the height. This it does approximately, but the presentation of a curve in demonstration of the fact seems unnecessary inasmuch as all the essential data have been already presented in tables above. (See table 4 for average heights.)

Strength-distribution. In addition to affording evidence that the strength tends to bear a fixed ratio to weight and a definite relationship

to the cube of the height, the data here under examination furnish a means of determining the average distribution of the strength among the muscle groups, in other words the "muscular symmetry." In an earlier section, as an aid to the calculation of total strength when some muscle-groups were not available for testing, the table of ideal strength-distribution is set down (table 2). The subject is reopened here in order to emphasize the possible significance of data of this kind, especially in connection with the interpretation of specific exercise effects or of limitations of activity due to habits or clothing (7). Obviously the possession of ideal standards of strength-distribution is the first requisite toward such interpretations. The particular standards here presented are based upon averages from a rather limited series of cases. For that reason they are subject to some modification as more data are accumulated. Their validity as standards must be established, furthermore, by the demonstration that they are truly representative, that the deviations of individual cases from the averages proposed as standards are not unduly wide. In the table of strength-distributions (table 2) standards for three age groups are submitted instead of standards for each age. Moreover, the same figures are proposed for given muscle-groups on the right and left sides of the body, notwithstanding that there is a slight general preponderance of strength on the right side. Although in making these changes certain errors are introduced, the practical advantage is so great, since the number of figures in the table is reduced from 588, the number when all ages and both sides of the body are included, to 63, the present number, that the errors are permissible provided they are not too great. The extent of error is indicated by a direct comparison of the substituted figures with those they are designed to replace. This comparison shows that 83 per cent of the 588 figures in the large table agree with the substituted figures within 10 per cent; all but 45 of the 588, or 93 per cent, agree within 15 per cent; and the average deviation of the entire series is only 6.2 per cent. Furthermore, more than half of the wide deviations (29 out of 45) are in the ages 13, 14, 15 and 17, in which, as table 1 shows, there were the fewest subjects and in which also the additional factor of differences in strength-distribution on account of sex might be expected to show if such differences exist.

The real test of the validity of the figures proposed as standards of strength-distribution must come from a comparison with them of the strength-distribution in individuals. This comparison has been made in all of the two hundred and forty cases making up this series. The

method used was to determine for each subject the percentage of his calculated total strength represented by each of his normal muscle-groups, and then to find the percentage deviation of each group from the standard as given in table 2. The mean deviation from standard of all the normal muscles in any individual gives an expression of his symmetry. Obviously, if his strength-distribution corresponds exactly with that given in table 2 for his age-group his mean deviation will be zero; the figure for mean deviation will become larger and larger as various muscle-groups differ more and more widely from the standard.

TABLE 6

Mean deviations from ideal symmetry. The numbers in the different columns stand for the number of cases whose mean deviations fell within the limits indicated at the head of the columns

AGE	MEAN DEVIATIONS NOT MORE THAN 12.5 PER CENT	MEAN DEVIATIONS BETWEEN 12.6 AND 17.5 PER CENT	MEAN DEVIATIONS BETWEEN 17.6 AND 22.5 PER CENT	MEAN DEVIATIONS MORE THAN 22.6 PER CENT
5	8	7	7	6
6	2	11	6	
7	8	12	14	3
8	5	15	10	2
9	6	14	8	3
10	6	5	6	
11	3	6	7	1
12	5	4	1	
13	4	3	2	
14		1	2	
15	1	2	3	1
16	2	6	2	1
17		4	2	2
18	3	4	2	2
Totals.....	53	94	72	21

Average deviation from ideal symmetry of entire series of 240 cases = 16.7 per cent.

For convenience of reference the observed mean deviations for the subjects of this study are tabulated in table 6. The range of deviations in the different columns was selected arbitrarily but with the idea of grouping the exceptionally symmetrical cases in one column, the fairly symmetrical in another, the somewhat unsymmetrical in a third and the definitely non-symmetrical in a fourth. The physical appearance of the subjects, as suggesting symmetry or non-symmetry, had some-

thing to do with the establishment of the limits of the different columns. Those whose mean deviations did not exceed 12.5 per cent were to the most casual inspection exceptionally symmetrical in bodily configuration. Those that fell in the second group, with mean deviations not exceeding 17.5 per cent, presented the physical appearance of symmetry, probably not distinguishable from the members of the first group except, perhaps, on the basis of actual measurements. The third group, consisting of subjects whose mean deviations fell between 17.6 per cent and 22.5 per cent, included many who were definitely recognizable as likely to be somewhat unsymmetrical; thus in this group were a number who were conspicuously strong for their age and size; others were as evidently weaker than normal; still others showed perceptible disproportion between arms and legs. All these conditions might tend toward diminished symmetry. The members of the fourth group, with few exceptions, were cases that might be picked out by any careful observer as unsymmetrical, except for three very young children, included in the list of 5-year-old cases, but actually only 4 years of age. It is probable that the tests on these very young children were less reliable than those of the older subjects. One 11-year-old girl and two boys, 16 and 18 years old respectively, gave tests which brought them into this group, without there being any obvious physical departure from symmetry. All the others of the fourth, or non-symmetrical group, were readily recognizable from mere inspection as belonging to it.

Included in columns 1 and 2 of table 6 are one hundred and forty-seven cases, reckoned in this classification as fairly or exceptionally symmetrical. These make up 61 per cent of the entire series of two hundred and forty cases. Included among the remaining 39 per cent or ninety-three cases, are eight that exceed the limit of column 2 by so narrow a margin that a change of 10 per cent in the right direction in the single muscle-group furthest removed from ideal symmetry would bring them into column 2, and at least thirty-five cases that for one reason or another would be expected to show considerable departure from ideal symmetry. The remaining fifty cases constitute a group of unexplained deviation from symmetry which is probably no larger than may be expected to appear in such a series as this. When we recall the nature of the test together with the readiness with which individual muscle-groups may be developed by special exercises and the likelihood that in any group of two hundred and forty children a number would be found who had been accustomed to using particular

muscle-groups to a greater degree than common, the percentage of satisfactorily symmetrical cases seems ample.

An additional check upon the validity of the proposed standards of strength-distribution is afforded by the study of individual muscle-group percentage distributions as distinct from the averaged records of all the tests upon single subjects. Since a mean deviation of 17.5 per cent was adopted as the upper limit of reasonable symmetry in the subject, a slightly higher deviation, 20 per cent, may be permitted in the individual muscle-group. Of the entire number of muscle-groups tested 70 per cent agreed with expectation within 20 per cent, the remaining 30 per cent diverging from expectation more widely than 20 per cent. In the case of individual muscle-groups "expectation" consists of the percentage figure for the muscle-group in question as set down in table 2. An agreement within satisfactory limits of 70 per cent of all the muscle-groups tested is probably as good as can be hoped for, considering the nature of the tests. So far as the material at hand is concerned there seems sufficient justification for the adoption of the average strength-distributions of table 2 as standards of symmetry in children.

DISCUSSION

The early part of this paper is devoted to an account of observations which show that in children the strength tends to bear a fixed relation to body-weight. In placing this interpretation upon the observations no violence is done to prevailing ideas. It is a commonplace that the strength of individual muscle-groups is determined largely by the use to which those groups are put. In cases where specific exercise effects are not present, which are precisely the sort of cases to which this discussion is intended to apply, the one element of muscle-use which is constantly operative and which enters as a factor in all muscular movements, is weight, either of the entire body or of the part of the body in which a particular muscle-group has insertion. The strength-weight ratios suggested by these observations are, of course, applicable only in connection with this special system of muscle-testing and they are subject to minor modification when larger series of tests are available, but the principle here advocated, that in the absence of specific exercise effects the body-weight is the determining factor of strength, seems worthy of serious consideration. If this principle can be definitely established we have a generalization on which to base expectations of

strength in individuals, and a point of departure from which to analyze the effects on strength of given amounts or kinds of exercise.

Similarly, as brought out above (p. 77), information as to the ideal distribution of strength among the muscle-groups may enable us to interpret and perhaps to regulate departures from the ideal due to special exercises or to particular habits. Standards of muscular symmetry may come to have a value comparable to that of standards of anatomical symmetry.

As a contribution toward the establishment of quantitative standards of physiological activities these observations are submitted.

SUMMARY

1. Strength tests by means of a spring-balance method were made on two hundred and forty children between the ages of 5 and 18 years.

2. The average percentage distribution of the strength among the muscles of the body is determined for each age. It is found that the percentage distribution of strength among the muscles can be stated for three age-groups, 5 to 7, 8 to 12, and 13 to 18 without introducing serious error. These percentage distributions (table 2) are proposed as standards of muscular symmetry in children.

3. The previous finding of Kellogg that there is relatively little difference in strength between the two sides of the body is confirmed.

4. Data are presented to show that calculations of entire strength based upon tests of only part of the muscle-groups in the body are valid within a reasonable margin of error.

5. The average strength for each age is found. It is somewhat less in females than in males.

6. The ratio of the average strength for any age to the average weight for the same age, keeping the sexes separate, is approximately constant. The value of this constant for males is 20 and for females 18. For males of 5 years or less the ratio is the same as for female children of all ages. Males above 16 years have a higher ratio than 20.

7. Application to individuals, regardless of their ages, of the above constants, shows that the ratios apply within a reasonable limit to 63 per cent of the cases examined.

8. The relation of strength to height is that which should follow from the principle that the mass varies as the cube of a linear dimension and from the demonstration that the strength varies directly as

the weight. There is no evidence that undue height tends to reduce the effective strength.

9. The fixed ratio of strength to weight is interpreted as signifying that the effective strength, as manifested in volitional efforts, depends, in the absence of specific exercise effects, upon the constantly operative factor of weight, either of the entire body or of the part moved by a particular muscle-group.

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THE REGULATION OF RENAL ACTIVITY

VI. The Effect of Adrenalin and Pituitrin on the Action of the Kidney under Strain[1]

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By the term strain is meant the condition imposed on the kidney when it is called on to increase its rate of work because of an increase in the concentration of urinary constituents in the blood. In the case of urea this condition may be induced at will by the administration of preformed urea. The following table shows how the rabbit's kidney reacts to the strain resulting from the introduction into the stomach of 5 grams of urea.

TABLE 1

Effect of strain on the urea excreting activity of the kidney

PERIOD	WITHOUT STRAIN (Averages of 35 experiments on 27 rabbits given no urea)			WITH STRAIN (Averages of 143 experiments on 51 rabbits given 5 grams urea)		
	Urea in 1 hour's urine	Urea in 100 cc. blood	Ratio:	Urea in 1 hour's urine	Urea in 100 cc. of blood	Ratio
	mgm.	*mgm.*		*mgm.*	*mgm.*	
I	62	70	0.79	232	133	1.82
II	94	70	1.25	417	220	1.98
III	106	73	1.38	481	236	2.08
IV	111	75	1.56	488	233	2.11

Both of the groups of animals compared in the above table were under exactly the same experimental conditions except that the rabbits in the larger group were given 5 grams of urea dissolved in 25 cc.

[1] A note on the effect of adrenalin and of pituitrin on the urea excreting function of the kidney under strain was published in the Proc. Soc. Exper. Biol. and Med., 1916, xlv, 49.

of water at the beginning of the experiment. The manner in which the kidney responded to the strain thus induced is shown in the rates of urea excretion for these periods. There is not only a marked increase in the amounts of urea eliminated, but this increase is relatively greater than the increase in the blood concentration, for while the urea in the blood is not much more than three times greater, the urea in the urine is well over four times larger in amount than in animals not given urea. Strain, therefore, is a condition under which the urea excreting activity of the kidney is not only absolutely but also relatively increased. The effort put forth by the kidney is more than sufficient to meet the increased demand for work. This state of renal hyperactivity is expressed in the greater magnitude of the ratio between the urea content of the urine and of the blood after urea administration.

It has been shown that the unknown factors in the regulation of renal activity whose nature we are investigating reveal their existence through the variations they induce in the rate of urea excretion, variations which cannot be accounted for as a result of changes in blood urea concentration. And it will be remembered that these variations become less marked the greater the strain on the kidney. For when the relative incidence of unexplained fluctuations in rate above and below the curve of the average rate was plotted on a scale according to the blood urea concentrations at which the rates were measured, a curve resulted which indicated that the variability of the rate was high at low blood concentrations but decreased as the blood urea concentration increased (1).

It has further been shown that at low blood concentrations all degrees of those variations in excess of the average rate, which at times and irregularly are observed under our standard conditions may be almost constantly and at will induced by the subcutaneous administrations of appropriate amounts of adrenalin (2). Similarly the variations below the curve of the average rate which appear spontaneously and occasionally in any series of observations may be duplicated experimentally by the injection of pituitrin (3).

It therefore becomes of interest to determine whether adrenalin and pituitrin have the further resemblance to the unknown factors of producing relatively smaller variations above and below the curve of the average rate of urea excretion when the kidney is under the condition we have defined as strain.

The effect of adrenalin and of pituitrin on the kidney subjected to

strain is shown in tables 2 and 3. They give the averages of groups of rabbits without and with adrenalin or pituitrin. Five grams of urea dissolved in 25 cc. of water were given to each animal at the commencement of the experiment.

It is evident that the effect of adrenalin and of pituitrin remains qualitatively the same under strain as under conditions where there is no strain. The ratio is still increased by adrenalin and decreased by

TABLE 2

Effect of adrenalin on the kidney under strain

PERIOD	WITHOUT ADRENALIN (Averages of 24 experiments on a group of 9 rabbits given 5 grams urea)			WITH 0.5 CC. ADRENALIN (Averages of 14 experiments on the same group of 9 rabbits given 5 grams urea)		
	Urea in 1 hour's urine	Urea in 100 cc. of blood	Ratio:	Urea in 1 hour's urine	Urea in 100 cc. of blood	Ratio:
	mgm.	*mgm.*		*mgm.*	*mgm.*	
I	195	131	1.68	273	94	2.96
II	468	232	1.98	509	171	2.96
III	481	249	1.99	506	174	2.95
IV	461	240	2.01	457	165	2.78

TABLE 3

Effect of pituitrin on the kidney under strain

PERIOD	WITHOUT PITUITRIN (Averages of 16 experiments on a group of 8 rabbits given 5 grams urea)			WITH 0.25 CC. PITUITRIN (Averages of 12 experiments on the same group of 8 rabbits given 5 grams of urea)		
	Urea in 1 hour's urine	Urea in 100 cc. of blood	Ratio:	Urea in 1 hour's urine	Urea in 100 cc. of blood	Ratio:
	mgm.	*mgm.*		*mgm.*	*mgm.*	
I	224	126	1.64	175	100	1.72
II	482	228	2.08	267	186	1.47
III	490	242	2.04	276	208	1.34
IV	496	229	2.18	271	221	1.27

pituitrin. The difference between the levels of blood urea concentration in the control as compared with the adrenalin and pituitrin experiments is probably due to a retarding effect of both these substances on the rate of absorption of the administered urea from the gastro-intestinal tract, yet even here the divergent effect on the kidney can be traced, for while the blood concentration tends to fall toward the end of the experiment in the adrenalin animals, it continues to rise progressively when pituitrin is given.

The point with which we are immediately concerned, however, is to determine whether the augmenting action of adrenalin and the depressing action of pituitrin is quantitatively different in degree under these conditions of strain as compared with the degree of their action under conditions which impose no extra work on the kidney. But the percentage of change in rates or ratios induced by adrenalin or pituitrin cannot be calculated from the averages of the control experiments because of the difference in the levels of blood urea concentration to which we have referred. These differences make the changes less than those which would have resulted had an equality of blood concentration been attained. It is, therefore, necessary to compare either the rates or

TABLE 4

Comparison of the percentage deviations from the average rate of urea excretion produced by adrenalin when acting on a kidney without strain and when acting on a kidney with strain

PERIOD	ADRENALIN 0.5 CC. WITHOUT STRAIN (Averages of 13 experiments on a group of 13 rabbits)					ADRENALIN 0.5 CC. WITH STRAIN Averages of 15 experiments on a group of 9 rabbits)				
	Blood urea concentration per 100 cc.	Average rate of urea excretion	Rate of urea excretion after adrenalin	Deviation from average rate	Percentage deviation	Blood urea concentration per 100 cc.	Average rate of urea excretion	Rate of urea excretion after adrenalin	Deviation from average rate	Percentage deviation
	mgm.	*mgm.*	*mgm.*	*mgm.*	*per cent*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*per cent*
I	42	30	54	+24	+80	94	160	273	+113	+71
II	48	45	82	+37	+82	171	332	509	+177	+53
III	50	58	104	+46	+79	174	380	506	+126	+33
IV	52	63	103	+40	+64	165	375	457	+ 82	+22

the ratios after adrenalin or pituitrin with the general average rate or ratio obtained at the same blood urea concentration under the standard control conditions. But this also would be a faulty method. For the average rate and ratio curves we have given (4) are composites of rates and of ratios during each of the four periods of the experiment. But we have shown that the rates and ratios increase progressively during each successive period, even though the blood urea concentration remains the same (1). The curve of the average is, therefore, different for each period. We had, however, determined the curve of the average rate of urea excretion for each period separately and we have accordingly taken these curves as a more correct standard for comparison

than the general curve we gave in our first paper. In tables 4 and 5 the percentage deviation produced by adrenalin and by pituitrin from the curve of the average rate for each period at the levels of blood urea concentration found when the kidney was not under strain, has been compared with the percentage deviation produced by the same amounts of adrenalin and of pituitrin from the curve of the average rate for each period at the levels of blood urea concentration found in the above experiments after the administration of urea.

TABLE 5

Comparison of the percentage deviations from the average rate of urea excretion produced by pituitrin when acting on a kidney without strain and when acting on a kidney with strain

PERIOD	PITUITRIN 0.25 CC. WITHOUT STRAIN (Averages of 9 experiments on a group of 9 rabbits)					PITUITRIN 0.25 CC. WITH STRAIN (Averages of 12 experiments on a group of 8 rabbits)				
	Blood urea concentration per 100 cc.	Average rate of urea excretion	Rate of urea excretion after pituitrin	Deviation from average rate	Percentage deviation	Blood urea concentration per 100 cc.	Average rate of urea excretion	Rate of urea excretion after pituitrin	Deviation from average rate	Percentage deviation
	mgm.	*mgm.*	*mgm.*	*mgm.*	*per cent*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*per cent*
I	66	65	20	−45	−69	100	175	175	− 0	− 0
II	68	75	35	−36	−48	186	365	267	− 98	−27
III	73	105	27	−47	−45	208	465	276	−189	−41
IV	77	105	33	−44	−42	221	502	271	−231	−46

These figures show that strain considerably reduces the degree of deviation from the average. With low blood urea concentrations the rate of urea excretion is 76 per cent higher with adrenalin when the average deviation for all four periods is taken. Under strain the increase is only 45 per cent. With pituitrin a decrease of 51 per cent without strain is reduced to a decrease of 29 per cent under strain.

Adrenalin and pituitrin, therefore, resemble the unknown factors concerned in the regulation of renal activity, not only in producing deviations both above and below the plane of activity usually found at any given blood urea concentration but also in producing a lesser degree of deviation when the kidney is under strain.

CONCLUSION

At high blood urea concentrations the degree of change in the urea excreting activity of the kidney produced by adrenalin and by pituitrin is less than at low blood urea concentrations.

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THE RELATION OF THE ADRENALS TO PIQÛRE HYPERGLYCEMIA AND TO THE GLYCOGEN CONTENT OF THE LIVER

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PART I. THE RELATION OF THE ADRENALS TO PIQÛRE HYPERGLYCEMIA

We have recently (1) studied the question whether the epinephrin secretion of the adrenals is indispensable for the production of certain experimental hyperglycemias. The majority of previous investigations have suffered from the defect that they were carried out, if not on practically moribund animals, at least on animals still under the effects of a serious operation. This undoubtedly is the chief reason for the astonishing lack of uniformity in the results. Working with animals (cats) in which the epinephrin secretion was abolished or reduced to an insignificant fraction of the normal by removal of one adrenal and section of the nerves of the other (an operation which does not preclude the continued life of the animal in good health), we were able to show that two forms of experimental hyperglycemia—that produced by ether and that produced by asphyxia—are as readily obtained in the absence of epinephrin secretion as when the adrenals have not been interfered with. We purposely reserved the question of the relation of the adrenals to piqûre hyperglycemia for further investigation since it is the form which is commonly supposed to be more closely associated than any other with the activity of the adrenal medulla. In his earlier work Kahn (2) was unable to find evidence of increased epinephrin liberation following piqûre. His later statement (3) that the piqûre causes an augmented epinephrin liberation, even if it were well founded, would by no means settle the question. For his observations furnish no evidence that the quantity liberated is at all comparable to the quantity required to produce the typical adrenalin hyperglycemia and glycosuria when adrenalin is artificially injected. But in fact Kahn's ex-

periments do not show that the output of epinephrin is at all influenced by the sugar puncture. Deductions from the relative depth of the coloration produced by chromium salts in the adrenal medulla have little or no quantitative value. And such estimations as he made by the Läwen method are vitiated by the fact that he took no account of changes in the rate of blood flow through the adrenals in the period for which the animal was allowed to survive following the piqûre. An increase in epinephrin concentration in the adrenal vein blood shown by the perfused frog preparation could not be interpreted as an increase in the output of epinephrin per unit of time unless it were known that the blood flow through the adrenals had not been proportionately diminished after the puncture, as compared with the flow during collection of the comparison samples before the puncture. Kahn did not even collect pure adrenal vein blood, but drew off blood from the inferior cava. The difference in vasoconstricting power of different samples of serum or defibrinated blood is also a factor which detracts greatly from the value of such estimations on blood-vessel preparations.

It has not been sufficiently recognized by some of the other investigators who have published experiments purporting to show an increased rate of liberation due to this or that factor that certain indispensable conditions must be fulfilled if the comparative tests are to have any quantitative value. When blood from the adrenals is withdrawn and then tested on such objects as rabbit intestine and uterus segments, it is practically always the concentration of epinephrin in the liquid which is estimated, since the concentration of adrenalin added to an indifferent specimen of the same blood which will give an effect equal to that of the adrenal specimen is determined. The condition of the segment in all observations which are to be compared must of course be approximately the same, as we have repeatedly pointed out in other papers. The concentration as thus estimated gives no information as to the rate of liberation of epinephrin per unit of time unless the mean rate of blood flow through the adrenals during collection of the given specimen is known.

When deductions are made in regard to the rate of liberation of epinephrin from experiments on test objects *in situ* it is of course just as necessary to see that in any observations compared the test object is in the same condition as regards reactivity, the rate of the blood flow through it, etc. For example, von Anrep (4) states that if the nerves of a hind limb or kidney be cut, these denervated parts respond to stimulation of afferent nerves (central end of sciatic) by a vasoconstric-

tion if the adrenals have not been interfered with, but only show the initial passive dilatation due to the rise of blood pressure if the adrenals have been extirpated. He draws the conclusion that stimulation of the sciatic reflexly increases the rate of liberation of epinephrin. But granting that the characteristic vasoconstriction can only be elicited with intact adrenals (and von Anrep's careful work seems to leave little doubt that this is a fact) this does not at all prove that during stimulation of the nerve more epinephrin is being poured into the blood per unit of time than without stimulation, for during stimulation of the sciatic the condition of the test object is greatly altered. The rise of blood pressure must necessarily increase the rate of blood flow through the denervated region. Usually the increase will be very considerable. With the adrenals intact and steadily discharging epinephrin at the normal rate, this means that the amount of epinephrin passing per unit of time through the vascular tract in question is abruptly and markedly augmented. If such denervated areas are as sensitive to epinephrin as is claimed, they may be expected to respond to this increase in the amount of epinephrin traversing them, even if no change whatever has taken place in the rate of its liberation from the adrenals. This reaction, accordingly, is not at all out of harmony with our observations made by a more direct method; namely, collection of adrenal vein blood during and without afferent nerve stimulation, and testing of the blood specimens on rabbit intestine and uterus segments. If afferent stimulation really causes such a great increase in the output of epinephrin as some authors believe, we ought to have been able to capture some of this epinephrin-rich blood coming from the adrenals, but we found no difference in the output in samples collected in equal times with and without afferent nerve stimulation (5). It is obvious that if the phenomenon studied by von Anrep is due to passage through the denervated region of an increased amount of blood with the ordinary content of epinephrin, it will necessarily be abolished by excision of the adrenal or by clipping of the adrenal veins, just as if it were due to a greatly augmented output of epinephrin produced reflexly. All that can be deduced from the fact that after elimination of the adrenals the vasoconstriction in the denervated part is absent, is that epinephrin was being given off from the adrenals, a fact which is undisputed.

We have shown (6) that as a matter of fact an increase in the amount of blood supplied to a test object *in situ* (denervated eye) without change in the concentration of the epinephrin, is associated with an increased epinephrin reaction, or with the appearance of a positive reaction where

none was elicited with the previous rate of blood flow. For example, if a strength and duration of stimulation of the peripheral end of one or of both splanchnics be sought which will just fail to cause dilatation of the pupil of the denervated eye, a definite dilatation will in general be obtained when certain alternative arterial paths are occluded at the proper time, so as to allow more of the epinephrin-containing blood to pass to that eye. The concentration of epinephrin in the blood will, of course, not be affected. A precisely similar result is obtained with artificially introduced adrenalin. Here it would be obviously absurd to say that the pupil reaction had been elicited because the rate of liberation of epinephrin from the adrenals or the rate of injection of adrenalin had been increased by clamping alternative paths at a time when the liberation or injection had been already completed.

Another, perhaps less important, factor may be involved in von Anrep's experiment, which likewise he has not taken into account. The reflex vasoconstriction associated with stimulation of the sciatic may be expected to cause, for a short time at least,—and this is all that concerns us,—a slackening in the flow through the splanchnic area and therefore a diminution in the quantity of blood coming to the inferior cava and mingling with the adrenal blood.[1] The epinephrin secretion proceeding unchanged at the normal rate, the concentration of epinephrin in the blood entering and leaving the heart will thus be increased, so that not only will the denervated region receive a much augmented quantity of blood, but the concentration of epinephrin in this blood may be greater than before the nerve stimulation without any increase having taken place in the output of epinephrin per unit of time.

Von Anrep himself performed an experiment, which we believe proves conclusively that the effects observed by him are not due to augmented epinephrin secretion but to a redistribution of the blood containing epinephrin given off at the previous steady rate. He says:

[1] The observations of Edwards (7) on the compensatory increase of blood flow through the head and limbs during stimulation of the splanchnic are not out of harmony with the supposition that stimulation of the central end of the sciatic may cause an increase of concentration of epinephrin in the blood of the inferior cava in the manner suggested without any change having occurred in the rate of liberation of epinephrin from the adrenals. For we do not know that a similar compensation occurs, or that it occurs as promptly with stimulation of the central end of a peripheral nerve trunk, which may cause vasoconstriction in the head and limbs also, as when the peripheral end of a splanchnic is stimulated.

It is important to note that if one splanchnic nerve is intact while the suprarenal on the other side is extirpated, stimulation of the splanchnic nerve on the side of the extirpated suprarenal may still cause constriction of the denervated limb. Only after the other splanchnic nerve is cut does this constriction disappear and the limb react passively to the changes of blood pressure.

His interpretation of this result is that

this is due to the fact that stimulation, even of the peripheral end (of the splanchnic), excites a certain number of afferent nerves, so that there may be a reflex excitation of the suprarenal of the other side through the intact splanchnic nerve.

There is no evidence that stimulation of the peripheral end of the splanchnic nerve can affect reflexly the rate of epinephrin secretion from the other adrenal, and we have good evidence against it. The true explanation, we believe, is that the liberation of epinephrin is proceeding steadily at a substantial rate from the adrenal with intact splanchnic and blood containing a concentration of epinephrin corresponding to this discharge is passing steadily through the denervated region as through the rest of the vascular system. Whatever influence it is exerting cannot of course be revealed till some change occurs in the concentration or in the amount passing through per unit of time. When the splanchnic on the side of the extirpated adrenal is stimulated, there is no increase in the rate of liberation of epinephrin from the remaining adrenal but the vasoconstriction in the splanchnic area causes a rise of arterial pressure which is straightway reflected in an increased flow of the epinephrin-containing blood through the denervated area. This area naturally responds, granting that such vascular regions are as sensitive to epinephrin as von Anrep assumes, by a vasoconstriction to the larger amount of epinephrin offered to it.

The similar reactions elicited by asphyxia, and obtained not at all or in smaller degree after elimination of the adrenals, in like manner afford no evidence in favor of a greatly augmented output of epinephrin, certainly no evidence which can be set up against such direct tests as we have made with the actual adrenal vein blood (10). In the case of asphyxia an additional and a formidable complication is introduced in such indirect experiments as those of von Anrep by the fact that asphyxia may be expected to alter the reactivity of the test object to epinephrin. Unless this factor is controlled, it is impossible to say that an increased reaction is due to an augmented liberation of epinephrin and not to an increased sensitiveness of the test object to such amounts as were already present.

When we have used as test objects organs or tissues *in situ* we have endeavored to render the observations to be compared really comparable by collecting the blood to be tested in the cava pocket and only releasing it when the test object was again approximately under the original conditions. For instance, in determining whether stimulation of afferent nerves caused any change in the rate of liberation of epinephrin, we collected blood in the pocket for a given time, then released it and noted the effect on the eye and blood pressure. Then blood was collected for the same length of time during nerve stimulation. In many of our experiments the splanchnic area and hind limbs were purposely tied off, so that the reflex rise of blood pressure was usually negligible. But where the nerve stimulation produced any considerable rise of blood pressure the stimulation was stopped some time before the opening of the pocket so that the tissues of the eye-ball and the blood vessels might, as far as practicable, have the opportunity of reverting to the same conditions as regards rate of blood flow, etc., as were present in the comparison observation. Only after this interval was the epinephrin-containing blood in the pocket released. In our observations on asphyxia a longer interval was allowed to permit the blood pressure to return to normal, the respiratory movements to diminish and the asphyxial products, to some extent at least, to disappear. We never supposed that it was permissible to use in one observation an asphyxiated test object and in the comparison observation the same object with unobstructed respiration; or to assume that if there was any difference in the reactions it must be due to a difference in the rate of output of epinephrin, the condition of the test object itself being of no moment.

Certain reactions (especially acceleration) of the heart, when isolated from extrinsic nervous influence by section of the vagi and excision of the stellate ganglia, have also been supposed to prove that the rate of output of epinephrin from the adrenals is greatly increased reflexly by stimulation of afferent nerves. It is not difficult, however, to see that the results of the study of these reactions by von Anrep and others must be interpreted precisely in the same way as the results on the denervated vascular areas, and yield no better evidence of reflexly augmented epinephrin secretion than the vascular reactions do.

Thus von Anrep (8), starting with the demonstration that stimulation of the peripheral end of the cut splanchnics is followed by the characteristic heart reactions if the adrenals are present, but not if they have been removed, is led to the generalization that

every rise of blood pressure brought about by the agency of the nervous system involves the coöperation of the chemical mechanism represented by the suprarenal glands.

And it is clear that whether the rise of pressure be brought about by direct stimulation of the efferent splanchnic fibers, by asphyxia or by stimulaton of afferent nerves, he had no other conception of this coöperation than that the nervous action on the cardiovascular system is accompanied by a nervous action on the adrenals which causes an increased outpouring of epinephrin.

Nobody doubts that when the peripheral end of the cut splanchnic is stimulated the liberation of epinephrin from the corresponding adrenal, which has been reduced by the nerve section from the normal spontaneous rate to zero, or at least greatly diminished, is augmented. Von Anrep is therefore entitled to conclude that in this case a part of the characteristic vascular and cardiac reactions is due to the abrupt increase in the epinephrin content of the blood. But it is one thing to use the well established fact that stimulation of the peripheral end of the splanchnic increases the output of epinephrin to explain a reaction of the denervated heart which can be shown to depend upon the secretion of epinephrin by the adrenals, and quite another thing to deduce from the occurrence of these reactions when afferent nerves are stimulated the conclusion that they must be due to an *augmented* output of epinephrin, merely because they cannot be obtained in the absence of the adrenals.[2] This conclusion could only be accepted if it were shown that the increase in the rate of blood flow through the coronary circulation associated with the rise of blood pressure is inadequate to produce the reactions. The increased blood flow must necessarily be accompanied by an increased supply of epinephrin to the heart vessels per unit of time, even if no increase has occurred in the rate of liberation of epinephrin from the adrenals, and the denervated heart, an exceedingly delicate test object for epinephrin, according to von Anrep, may be expected to respond just as if the epinephrin output had been increased without any material change in the rate of flow. The possibility is also

[2] In our experience, in the cat (von Anrep worked with dogs) a not inconsiderable acceleration of the denervated heart can usually be obtained by stimulating the central end of the sciatic or the peripheral end of the splanchnic, even when the adrenal veins are clipped. There is nothing strange about this. It is obviously dependent upon the better blood flow through the coronary vessels. Guthrie and Pike (9) showed that in the perfused mammalian heart the rate could be increased decidedly by increasing the pressure of the perfusion fluid.

present, of course, in the case of the heart as in the case of the vascular reactions, that the concentration of epinephrin may be increased by stimulation of the central end of a peripheral nerve without any increase in the rate of the discharge per unit of time.

The question of the physiological value attributed by von Anrep to the reactions of epinephrin which he has so carefully studied, especially the question of the physiological value of the heart reactions is unaffected by substituting for the supposed reflex augmentation of the output of epinephrin an automatic redistribution of the blood which, without any material change in the rate of output, carries with it an increased supply of epinephrin to organs not involved in the vasoconstriction associated with the rise of blood pressure. We suggest that in such redistributions of blood containing the epinephrin secreted by the adrenals at a relatively stable and constant rate, rather than in a sudden outpouring of epinephrin, is to be sought the mechanism of any physiological effects of this type exerted by the naturally secreted epinephrin on the organism.

To return to the question of the relation of the adrenals to piqûre hyperglycemia, Kahn and Starkenstein (11) availed themselves in some experiments of the now well established fact that a certain proportion of rabbits deprived of both adrenals survive for a long time or indefinitely, in good health. In our experience the proportion is something like 20 per cent. If the animals which survive some weeks be included, it is greater. The statement of Freund and Marchand (12) that after complete extirpation of both adrenals rabbits die without exception in a short time, and usually on the first day, must be based on some error of operative technique. Unfortunately Kahn and Starkenstein contented themselves with tests, mostly qualitative, for sugar in the urine. They made no quantitative estimations of the blood sugar which in an investigation of this sort cannot be satisfactorily replaced by the qualitative tests on the aqueous humor employed by Kahn. Also in some of the very few relevant protocols published by them it is seen that practically no urine was secreted after the piqûre, and a negative sugar test in such cases would of course possess no value. It cannot therefore be admitted that the negative results of piqûre obtained by these writers on rabbits deprived of the adrenals have by any means settled the question of the indispensability of the epinephrin secretion for piqûre hyperglycemia.

The experiments of Biberfeld (13) are even less convincing and he admits that he does not now think that observations on sugar in the

urine, the only observations he made, are satisfactory in the absence of blood sugar estimations.

The investigations of Jarisch (14) are free from this objection but are nevertheless open to criticism for other reasons. He endeavored to show that in rabbits after complete section of all the possible nervous paths from the central nervous system to the liver, piqûre was still followed by hyperglycemia when nervous connections of one adrenal were left whereas it did not cause hyperglycemia when the innervation of the adrenals was completely severed, that of the liver being intact. He estimated the blood sugar by Bertrand's method, precipitating the proteins by Schenck's method. Unfortunately he contented himself with a single blood sample, taken some time after piqûre. He compared the sugar content of this sample with a theoretical normal level and not, as we have invariably done, with that of a preliminary sample taken from the same animal. This renders the classification of some of the results as hyperglycemia quite arbitrary. In one series of ten experiments, for example, in which the innervation of the left adrenal was preserved while the remaining splanchnic distribution was severed, he counts a blood sugar content of 0.164 per cent among the hyperglycemias, very likely quite correctly. This is the series of experiments designed to show that augmented epinephrin secretion can cause hyperglycemia without the intervention of the hepatic nerves. In another series of five experiments in which the right adrenal was extirpated and all the nerves of the left adrenal cut, he classifies a blood sugar content of 0.167 per cent not as a hyperglycemia but as at "the upper limits of the normal content." This series was designed to show that in the absence of epinephrin secretion by the adrenals piqûre does not cause hyperglycemia. It must further be objected that in very few of Jarisch's experiments was the interval after the primary operation sufficiently long to permit a great accumulation of glycogen in the liver, even although the animals received cane sugar by stomach tube some time before the piqûre was made. The proof of this is the very low glycogen content of the liver in most of the experiments, even making allowance for the loss of glycogen in successful piqûre observations. For example, in three experiments in which he states that there was no hyperglycemia and in which therefore the glycogen content of the liver at the end of the experiment probably did not differ much from the content just before the piqûre, the glycogen only amounted to 0.7, 1.1 and 1.3 per cent, respectively. In five of the experiments with hyperglycemia in which the liver glycogen was estimated at the end, the per-

centages were 0, 0.2, 0.3, 0.8 and 2.1. With a good initial content of glycogen in our experiments the residual content after hyperglycemia produced by piqûre and later on by asphyxia was much greater, although the animals were not killed for a considerably longer time after the piqûre than appears to have been the case in the experiments of Jarisch.

The series of five experiments which are supposed to prove the inefficiency of the piqûre when the adrenals have been denervated although the liver nerves are intact is, we believe, completely vitiated on account of the low initial glycogen content. The percentages at the end of the experiments were 0, 0.5, 0.8, 0.9, 1.4. If there was no hyperglycemia, as Jarisch concludes, these percentages are probably not very different from the percentages before piqûre. It is vain to argue on the basis of one or two control experiments that with 1 per cent of glycogen in the liver piqûre will normally succeed. In our experience there is considerable variability in this matter in normal rabbits and there does not seem to be any reason why the production of a distinct piqûre hyperglycemia should depend solely upon the percentage of glycogen in the liver. At least one other factor, the rate of consumption of sugar in the animal, is obviously concerned. Even as regards the rate of mobilization of the glycogen it is improbable that the percentage of this substance in the liver should be the sole determining factor, and it cannot be assumed that every animal will respond in the same way to a given procedure when the glycogen store reaches a given level. The only way to be sure that a negative result is not due to too small a glycogen store is to work with animals whose livers are well filled with glycogen, and then always to make a glycogen estimation. It is impossible to decide beforehand that this or that animal will have enough glycogen to render a positive result certain after piqûre, particlarly when the animal has been recently subjected to a major operation. It may also be pointed out that positive results are much more important than negative ones in a question of this kind, and that only a decided hyperglycemia should be accepted as a positive result. Negative results in animals whose glycogen content is low ought not to be used at all.

A further criticism of these experiments is that it is surely difficult to be certain that the whole nerve supply of the liver has been divided by such an operation as that practiced by Jarisch. Finally, he made the piqûre under ether anesthesia. Even if the anesthetic was administered only during the operation, how is one to be certain that a subsequent hyperglycemia was not due to the anesthesia rather than to

the piqûre? We have already shown (15) that in cats a brief administration of ether is capable of causing distinct hyperglycemia after the secretion of epinephrin has been abolished.

Among recent workers who have denied the importance of the adrenals in puncture hyperglycemia or glycosuria may be mentioned Wertheimer and Battez, Freund and Marchand and Trendelenburg and Fleischhauer. Wertheimer and Battez (16) found glycosuria in three cats following piqûre after removal of both adrenals. The experiments, however, were necessarily acute. In one case the animal was anesthetized with chloroform and, as the authors point out, it was impossible to discriminate between the effect of the anesthetic and the effect of the piqûre. In the other two cats spinal anesthesia (cocain) was employed. In five cats no definite glycosuria could be demonstrated. Blood sugar estimations were not made.

Freund and Marchand (17) conclude that the influence of piqûre is exerted directly on the liver and not through the adrenals. However, although they made numerous experiments on rabbits, none of them are entirely satisfactory. All were acute experiments, the piqûre being performed two or three hours after the removal of the adrenals, and it is difficult if not impossible in many cases to disentangle any effect of piqûre on the blood sugar from the effects of the operation and the anesthetic. The blood sugar was estimated by Bang's micro-method.

Trendelenburg and Fleischhauer (18) reached the conclusion that puncture glycosuria is not due to a "hormone" action of adrenalin discharged from the adrenals, since the rate of the discharge is not increased by the puncture. This result, however, is not arrived at by direct assay of the epinephrin coming from the adrenals, but is deduced from the fact that sugar puncture does not cause, in an anesthetized animal, a rise of blood pressure, whereas the minimal quantity of adrenalin which must be injected into a vein in order to elicit adrenalin glycosuria causes a distinct increase of blood pressure. Jarisch (14) has criticised, justly in our opinion, the deductions of Trendelenburg and Fleischhauer. For one thing, they relied entirely upon testing for sugar in the urine and did not estimate the blood sugar, the really important point. Their main argument is based upon premises by no means beyond question, and we believe that although their conclusion—that it is not an augmented epinephrin secretion which is responsible for puncture glycosuria—is correct, this cannot be established by such experiments.

Our own experiments were made on rabbits. The adrenals were removed at separate times. The interval between the first and second operation varied from eleven days to eight months. The piqûre was made ten days to eighty-one days after removal of the second adrenal. The floor of the fourth ventricle was exposed according to Eckhard's method, under local anesthesia by ethyl chlorid. A sample of blood was taken, usually from an ear-vein, before piqûre; a second sample about one to one and one-half hours after piqûre. About an hour later a third sample was drawn in order that some idea of the duration of the hyperglycemia and the maximum blood sugar content attained might be gotten. Finally asphyxia was produced by covering the mouth and nose at intervals for a period of fifteen to twenty minutes. The effect of the asphyxia on the heart-beat was sedulously controlled and a few free respirations allowed as soon as the heart was distinctly slowed, so that the asphyxia was never pushed to the point where life was in danger. A blood specimen was then drawn. The object of the asphyxia was to test whether, in the event of the piqûre yielding a negative result, a decided hyperglycemia was capable of being produced in any particular animal. We have already shown (15) that in cats with one adrenal removed and the secretion of epinephrin from the other abolished by section of its nerves, asphyxia invariably causes hyperglycemia when the glycogen content of the liver is not deficient. The animal was then killed and the glycogen content of the liver estimated by Pflüger's method, the sugar after hydrolysis of the glycogen being determined by Bertrand's method. The blood sugar was estimated by the method of Lewis and Benedict. Pearce's autoclave modification and the graduated test-tubes used by us in our previous work (1) were employed. It was recognized, of course, that when hyperglycemia had been produced by any of the procedures employed, the glycogen content as determined at the end of the experiment must be materially less than would have been found had the animal been killed at once. Accordingly before undertaking the piqûre experiments, we made a series of observations on the glycogen content of the liver under various diets, in cats whose epinephrin output had been interfered with by the operation mentioned and in rabbits and rats which had survived the removal of both adrenals. These experiments will be given in the second part of the paper.

The following condensed protocols illustrate the effects of piqûre and asphyxia on the blood sugar content in rabbits deprived of the adrenals.

Protocol. Rabbit 155, male

February 15, 1917. Left adrenal excised.

June 8, 1917. Weight, 2.625 kgm.

September 13, 1917. Right adrenal excised. From this time fed daily with carrots in addition to the ordinary diet (oats and hay daily, a carrot or a small quantity of green food once a week).

November 2, 1917. Weight. 3.275 kgm.

11.30 a.m. Obtained from ear normal blood specimen. It contained 0.102 per cent dextrose.

11.45 a.m. Piqûre.

12 30 p.m. Blood specimen from ear contained 0.205 per cent dextrose.

1.50 p.m. Blood specimen from ear contained 0.134 per cent dextrose. Asphyxia for 25 minutes, then at

2:20 p.m. Obtained blood from external saphenous vein, containing 0.216 per cent dextrose.

2.20 p.m Killed by heart-stab, removed liver. Glycogen in liver, 7.40 per cent.

Autopsy. Accessory adrenals not found. First piqûre a little above calamus and slightly to left of mid line; second piqûre about 10 mm. below the opening of the iter in mid line.

Protocol. Rabbit 181, male

November 19, 1917. Right adrenal excised.

November 30, 1917. Left adrenal excised. Weight 2.66 kgm. Some cane sugar was added to the drinking water from this date on. Otherwise, the ordinary diet.

February 19, 1918. Weight, 2.48 kgm.

12.30 p m. Normal blood specimen from ear contained 0.119 per cent dextrose.

12.50 p.m. Piqûre.

2.40 p.m. Blood from external jugular vein contained 0.349 per cent dextrose.

4.00 p.m. Blood from external jugular vein contained 0.449 per cent dextrose. Now caused asphyxia for 20 minutes and at

4.30 p.m. Blood obtained by cutting neck vessels, contained 0.517 per cent dextrose.

Liver at once excised; it contained 2.44 per cent glycogen. Taking the weight of the liver as 60 grams and the weight of the blood as 200 grams, the amount of glycogen which must have been mobilized merely to raise the blood sugar content to 0.52 per cent would correspond to 1.3 per cent of the liver weight. Therefore the initial content of glycogen must have been at least 4 per cent and no doubt considerably more.

Autopsy. Accessory adrenals not found. The piqûre was about 6 mm. above the calamus in the mid line.

Protocol. Rabbit 188, female

November 19, 1917. Excised right adrenal.

November 26, 1917. Gave birth to five young.

February 13, 1918. Excised left adrenal. Weight, 2.2 kgm.

Diet, cane sugar in drinking water and carrots given daily for four weeks prior to experiment, in addition to the ordinary diet.

March 12, 1918. Weight, 2.12 kgm.

10.40 a.m. Normal specimen from the ear contained 0.102 per cent dextrose.

11.00 a.m. Piqûre.

12.10 p.m. Blood from external jugular vein contained 0.161 per cent dextrose. Voided urine. Test with Fehling negative.

12.55 p.m. Blood from external jugular vein contained 0.176 per cent dextrose. Now caused asphyxia for twenty minutes.

1.15 p.m. Blood from jugular vein contained 0.262 per cent dextrose. Liver at once excised. It contained 2.35 per cent glycogen.

Autopsy. Piqûre 4 mm. above calamus in mid line. No accessory adrenals found in abdomen.

In these animals a decided hyperglycemia was caused by piqûre. The same is true of asphyxia following the piqûre. In the first experiment the piqûre hyperglycemia had distinctly diminished before asphyxia was induced, and after asphyxia the blood sugar content rose to fully the maximum level obtained in the first specimen after piqûre. In the second experiment, the second blood specimen taken after piqûre contained 0.1 per cent more dextrose than the first piqûre specimen. In spite of the high grade of the piqûre hyperglycemia, the blood sugar content increased still further during asphyxia. In the third experiment where the hyperglycemia after piqûre, although quite distinct, was not so great as in the second, the specimen drawn after asphyxia also showed a decided increase in the blood sugar as compared with the second specimen after piqûre. There can be no question, then, that piqûre, like asphyxia, is capable of causing hyperglycemia in rabbits after removal of the adrenals. Obviously, as already pointed out, in a question of this kind positive results are much more important than negative ones. In the three animals the liver was well filled with glycogen, a considerable period having elapsed since the last operation.

Negative results in animals taken at too short an interval after the adrenal operation and containing little glycogen in the liver, have of course no weight at all. Not a few of the observers who have denied the possibility of producing hyperglycemia by piqûre after adrenalectomy have been misled by want of attention to this point. The following protocols are samples of our negative experiments on adrenalectomized rabbits:

Protocol. Rabbit 156, male

March 20, 1917. Left adrenal excised.

June 8, 1917. Weight, 1,775 kgm.

September 15, 1917. Right adrenal excised. From this time on the animal was fed regularly with carrots in addition to the ordinary diet.

November 13, 1917. Weight, 2.45 kgm.

11.00 a.m. Normal blood specimen from ear contained 0.101 per cent dextrose.

11.35 a.m. Piqûre. Two stabs.

12.15 p.m. Blood from cut external saphenous vein contained 0.107 per cent dextrose.

1.10 p.m. Blood from femoral vein contained 0.097 per cent dextrose. Now caused asphyxia for twenty minutes and at

1.40 p.m. Obtained blood specimen from external jugular vein, containing 0.121 per cent dextrose.

Immediately killed and excised liver, which contained 1.02 per cent of glycogen.

Autopsy.—Accessory adrenals not found. The first piqûre was 3 to 4 mm. above the calamus in the mid line, the second 6 to 7 mm. above the first in the mid line.

Protocol. Rabbit, 183, male. Weight, 2.235 kgm.

December 7, 1917. Right adrenal excised. Weight, 2.24 kgm.

Feb. 11, 1918. Left adrenal excised. Weight, 2.20 kgm. Kept on ordinary diet from this date, except that some cane sugar was given in the drinking water one day before the piqûre experiment. The weather was very cold at this time.

February 21, 1918. Weight, 2.235 kgm.

11.00 a.m. Normal blood specimen from ear contained 0.114 per cent dextrose.

11.10 a.m. Piqûre.

12.10 p.m. Blood specimen from ear contained 0.131 per cent dextrose.

1.20 p.m. Blood from ear contained 0.121 per cent dextrose. Asphyxia was now caused for twenty-five minutes, then at

1.50 p.m. Obtained blood specimen from external jugular, containing 0.128 per cent dextrose.

The neck vessels were now severed and a blood specimen obtained which contained 0.126 per cent dextrose. The liver was at once excised; its glycogen content was 2.32 per cent.

Autopsy. A small accessory adrenal was found under the left renal vein. The piqûre was 6 to 7 mm. above the calamus in the mid line.

It would not be profitable to speculate on the reason for the absence of piqûre hyperglycemia in these animals. Since precisely similar negative results may be obtained in normal animals, we do not see how they can be connected with the presence or absence of the adrenals. While everybody is agreed that a high glycogen content is favorable for the occurrence of piqûre hyperglycemia, and that a very low glycogen content is incompatible with it, there is no evidence as already

stated, that hyperglycemia must necessarily be obtained in an animal with more than a certain percentage of glycogen in its liver, whereas in animals with less than that percentage it can never be obtained.

The following protocols illustrate the results on normal rabbits with the same technique as that employed for the adrenalectomized animals:

Protocol. Rabbit 186

Male, which had been long in stock. Weight, 1.80 kgm. Ordinary diet.
February 27, 1918.

11.00 a.m. Normal blood specimen from ear contained 0.107 per cent dextrose.
11.15 a.m. Piqûre.
12.15 p.m. Blood from ear contained 0.374 per cent dextrose.
1.20 p.m. Blood from ear contained 0.46 per cent dextrose. Asphyxia was now caused for twenty-five minutes and at
1.45 p.m. A blood specimen was obtained with 0.514 per cent dextrose.

Ten minutes later the blood vessels in the neck were severed and another blood specimen obtained with 0.52 per cent dextrose. The liver was immediately excised. Its glycogen content was 2.96 per cent. The liver weighed 61 grams. Taking the total blood as 140 grams, the amount of glycogen which must have been mobilized merely to raise the blood sugar to 0.52 per cent would represent almost 1 per cent of the liver weight. The glycogen content before piqûre must therefore have been at least 4 per cent and no doubt was considerably more.

Autopsy. The piqûre was 5 mm. above the calamus in the mid line.

Protocol. Rabbit 182

Normal female from the stock. (Control for rabbit 181, and on the same diet).
February 19, 1918. Weight, 2.63 kgm.

12.10 p.m. Normal blood specmen from ear contained 0.11 per cent dextrose.
12.30 p.m. Piqûre.
1.40 p.m. Blood from ear contained 0.367 per cent dextrose.
3.00 p.m. Blood from ear contained 0.308 per cent dextrose. Asphyxia was now caused for twenty minutes and at
3.20 p.m. A blood specimen obtained from the ear contained 0.367 per cent dextrose.

The animal was now bled from the throat and a specimen obtained at 3.30 p.m. with 0.417 per cent dextrose. The glycogen content of the liver was 2.04 per cent. If only the amount of glycogen which must have been mobilized to make up a blood sugar content of 0.42 per cent be added, the liver must have contained before the piqûre experiment at least 3 per cent of glycogen and doubtless it contained more.

Autopsy. The piqûre was 4 to 5 mm. above the calamus in the mid line.

The following two protocols illustrate the negative results of piqûre obtained on normal rabbits just as on adrenalectomized rabbits.

Protocol. Rabbit 187, male

March 11, 1917. Weight, 1.41 kgm. No food given (except water) for two days prior to experiment.

10.00 a.m. Normal specimen of blood from ear contained 0.124 per cent dextrose.
10.15 a.m. Piqûre
11.15 a.m. Blood from external jugular vein contained 0.136 per cent dextrose.
12.15 p.m. Blood from external jugular vein contained 0.130 per cent dextrose. Now caused asphyxia for twenty minutes.
12.35 p.m. Blood from external jugular vein contained 0.142 per cent dextrose.

Severed blood vessels in neck and bled to death. A specimen of this mixed blood contained 0.151 per cent dextrose. The liver was at once excised; it contained 0.34 per cent glycogen.

Autopsy. The piqûre was 6 mm. above the calamus in the mid line.

Protocol. Rabbit 157

Normal female from stock. It had carrots regularly in addition to the ordinary diet for two weeks before the piqûre experiment.

November 13, 1917. Weight, 2.0 kgm.

11.15 a.m. Normal blood specimen from ear contained 0.122 per cent dextrose.
11.50 a.m. Exposed and opened the occipito-atlantoid membrane under local ethyl chlorid anesthesia, as in all the other experiments; but did not perform piqûre as yet.
12.40 p.m. Blood specimen fom ear contained 0.116 per cent dextrose.
12.55 p.m. Piqûre made (two stabs).
2.10 p.m. Blood from femoral vein contained 0.10 per cent dextrose. Asphyxia was now induced for twenty minutes and at
2.30 p.m. A blood specimen was obtained from the external jugular vein with 0.101 per cent dextrose.

The animal was at once killed by heart stab. The liver contained only a trace (less than 0.05 per cent) of glycogen.

Autopsy. First piqûre, 3 to 5 mm. above the calamus in the mid line, second piqûre, 7 to 8 mm. above the first and a little to the left of the mid line.

The object of the experiment just cited was to control the effect of the operation as such, apart from the piqûre, on the blood sugar. The experiment failed for this purpose because of the low glycogen content of the liver (in spite of the carrot diet) which did not permit either the subsequent piqûre or asphyxia to cause any hyperglycemia. The following protocol gives the data of an experiment of the same kind, but on a rabbit whose liver was well filled with glycogen. It will be seen that the operation as such, with any attendant emotional excitement, caused no hyperglycemia.

Protocol. Rabbit 189, male

Diet. Sugar in drinking water and carrots for five days prior to the experiment, in addition to the ordinary diet. The animal took the sugared water readily. Weight 2.0 kgm.

9.55 a.m. Normal blood specimen from ear vein contained 0.126 per cent dextrose.

10.15 a.m. Under local anesthesia (ethyl chlorid) exposed floor of fourth ventricle but did not perform piqûre as yet. Sutured the wound.

11.20 a.m. Blood specimen from ear vein contained 0.124 per cent dextrose.

11.30 a.m. Piqûre performed.

12.30 p.m. Blood specimen from ear vein contained 0.249 per cent dextrose. Asphyxia was now caused for twenty minutes and at

1.00 p.m. A blood specimen was obtained from the external jugular vein. It contained 0.343 per cent dextrose. Urine voided at this time gave a marked reduction with Fehling's solution. The animal was killed by bleeding from the neck vessels. The liver, excised immediately, weighed 82 grams and contained 7.14 per cent of glycogen.

Autopsy. The piqûre was 4 mm. above the calamus in the mid line.

The results of a number of preliminary experiments on normal rabbits in which glycogen determinations were not made, are given in table 1, in order to emphasize the point that a negative piqûre experiment in an adrenalectomized animal must not be attributed off hand to the absence of the adrenals. The six rabbits had lived a long time in the laboratory under identical conditions and on the same (ordinary) diet. Carrots were added one day before the experiment. It will be seen that three of the animals yielded distinctly positive results with piqûre. In two the result was negative, in one doubtful.

TABLE 1

Percentage of blood-sugar

NORMAL	PIQÛRE	ASPHYXIA
0.135	0.162	0.163
0.125	0.390	
0.130	0.134	
0.110	0.263	
0.101	0.178	
0.102	0.115	0.122

TABLE 2

Adrenalectomized rabbits

DATE OF GLYCOGEN ESTIMATION	NUMBER OF ANIMAL	ADRENALS EXCISED		PERCENTAGE OF GLYCOGEN IN LIVER	REMARKS
		First	Second		
1917 October 23	145	*1917* January 18	*1917* September 17	6.42	Carrots daily since last operation in addition to usual diet*
November 2	155	February 15	September 13	7.40	Same as for 145
November 13	156	March 20	September 15	1.00	Same as for 145
November 5	158	*1916* December 7	September 22	0.68	Attempted piqûre. Died; liver taken after one-half hour
1918 February 19	181	*1917* November 19	November 30	2.44	Cane sugar daily since last operation, in addition to ordinary diet
February 21	183	December 17	*1918* February 11	2.32	Cane sugar one day before experiment (ordinary diet). Very cold weather
February 25	184	December 7	February 13	Trace	Ordinary diet. Rabbit has mange, not eating well, losing weight
March 12	188	November 19	February 13	2.35	Cane sugar and carrots daily since last operation, in addition to ordinary diet

* Rabbit 145 was sacrificed for glycogen estimation. In all the others a piqûre experiment was done before the liver was excised. The ordinary diet for rabbits consisted of oats and hay daily, with a carrot or a small piece of green food once a week.

PART II. RELATION OF THE ADRENALS TO THE GLYCOGEN CONTENT OF THE LIVER

Several of the writers on the problem of the possibility of producing experimental glycosurias and hyperglycemias after removal of the adrenals have raised the question whether the negative result might not be due to inability of the liver to form or to store glycogen in the adrenalectomized animal. Since as shown above the result is not negative, this question does not arise. Nevertheless, before making our observations on piqûre and in the course of them, we made a con-

TABLE 3

Normal control rabbits

DATE	NUMBER OF ANIMAL	GLYCOGEN PERCENTAGE IN LIVER	REMARKS
1917			
October 23	144	2.80	Ordinary diet
October 29	150	4.73	Ordinary diet with addition of carrots for 6 days prior to experiment
November 22	160	2.58	Ordinary diet with addition of carrots for one week prior to experiment
November 13	157	Trace*	Ordinary diet with addition of carrots for one week prior to experiment. (Piqûre, etc.)
1918			
February 19	182	2.04	Cane sugar daily in addition to ordinary diet since November 30, 1917 (Piqûre, etc.)
February 27	185	1.74	Ordinary diet. Piqûre attempted. Died
February 27	186	2.96	Ordinary diet. (Piqûre, etc.)
March 11	187	0.34	Starved two days. Piqûre (negative)

* Less than 0.05 per cent.

siderable number of experiments on cats, rabbits and rats, in order to obtain information upon the glycogen content of the liver in animals in which the adrenals had been removed or, in the case of the cats, the secretion of epinephrin interfered with. The rabbits used had survived the removal of the second adrenal eleven days to nine months, when killed for the glycogen determination. Details as to diet, glycogen content, etc., are given in table 2, which includes a certain number of animals in which the glycogen was determined after hyperglycemia had been produced by piqûre and by asphyxia (see protocols in part I.). The glycogen, estimated at the end of these experiments must, of course, be less than the actual content before the piqûre.

In table 3 are displayed the results of a number of glycogen determinations on normal control rabbits. It will be seen that there is no material difference between the results in table 2 and table 3.

Since cats do not survive the removal of both adrenals, we excised one adrenal and divided the nerves of the other so as to abolish or reduce greatly the output of epinephrin. Protocols of two such experiments follow:

Protocol. Cat 125, male

Diet Liver, milk, occasionally fish and a daily ration of rice or potatoes boiled with milk.

August 18, 1917. Weight, 2.64 kgm. Excised right adrenal. It weighed 0.266 gram and contained 0.12 mgm. epinephrin. Extirpated left semilunar ganglion. Excised left superior cervical ganglion.

September 18, 1917. Weight, 1.92 kgm. Left pupil contracted and nictitating forward.

11.25 a.m. Urethane 3 grams (stomach).

2.50 to 3.15 p.m. Inserted tracheal and jugular cannulae; artificial respiration; coeliac and mesenteric arteries tied and a lobe of liver at once excised for glycogen estimation. Completed cava pocket in usual manner.

3.15 p.m. Both pupils equal and nictitating membranes slightly forward.

3.20 p.m. Pocket experiment two minutes. No eye reactions.

3.24 p.m. Pocket experiment five minutes. No eye reactions.

3.30 p.m. Pocket experiment nine minutes. No eye reactions.

3.44 p.m. 0.5 cc. 1: 1,300,000 adrenalin. Very good pupil dilatation in 11.2 seconds. No nictitating reaction.

3.48 p.m. 0.5 cc. 1: 2,600,000 adrenalin injected. Good pupil reaction in 11.4 seconds. No nictitating reaction.

3.53 p.m. 0.5 cc. 1: 4,000,000 adrenalin injected: Small pupil reaction in 13.8 seconds. No nictitating reaction.

3.58 p.m. 0.5 cc. 1: 5,200,000 adrenalin injected. Small pupil reaction in 13.6 seconds. No nictitating reaction.

Now collected the following blood samples from the adrenal (short pocket):

Sample 1, 1.4 grams in one minute (1.4 grams per minute).

Sample 2, 4.4 grams in four minutes (1.1 grams per minute).

Sample 3, 3.8 grams in eight minutes (0.475 gram per minute).

Obtained blood from abdominal aorta. Left adrenal weighed 0.257 gram, and contained 0.17 mgm. epinephrin. Glycogen in lobe of liver removed at beginning of experiment, 4.26 per cent.

The epinephrin assay of the adrenal vein blood gave the following results:

The tests with rabbit intestine and uterus segments showed that even the third adrenal specimen of blood could not have contained more than 1: 260,000,000 epinephrin, corresponding to an output of

0.0000018 mgm. per minute for the animal or 0.0000007 mgm. per minute per kilogram of body weight; i.e., not more than one-three hundred and fiftieth of the normal output as estimated by the segments.

The second adrenal specimen did not give a much greater effect than the indifferent arterial blood and a far smaller effect than indifferent blood containing 1: 260,000,000 adrenalin.

The eye reactions were negative even when blood was collected in the cava pocket for nine minutes. Yet distinct reactions were obtained when 0.5 cc. of a 1: 5,300,000 solution of adrenalin was injected. Accordingly, as tested in this way, the output could not have amounted to 0.00001 mgm. per minute for the animal or 0.000004 mgm. per kilogram of body weight per minute; i.e., not one-one hundred and fiftieth of the normal, as estimated by eye reactions.

Protocol. Cat 113, male

Same diet as for cat 125.

July 20, 1917. Weight, 2.725 kgm. Excised right adrenal. It weighed 0.152 gram and contained 0.16 mgm. epinephrin. Extirpated left semilunar ganglion and severed lumbar chain below the diaphragm.

August 16, 1917. Weight, 2.545 kgm. Right splanchnics divided.

August 30, 1917. Weight, 2.71 kgm. Blood sugar tests made as follows: Normal specimen, 0.089 per cent; specimen collected after frightening, 0.084 per cent; specimen collected after asphyxia, 0.153 per cent.[3]

August 31, 1917. Excised left superior cervical ganglion.

September 18, 1917. Weight, 2.41 kgm. Condition excellent.

10.15 a.m. 5 grams urethane (stomach).

11.50 a.m. Inserted tracheal and jugular cannulae; tied coeliac and mesenteric arteries; at once clamped off and removed the left and part of the middle lobe of the liver for glycogen estimation. Then completed a cava pocket in the usual way.

12.20 p.m. Left pupil wider than right; both nictitating slightly forward.

12.20 p.m. Pocket experiment two minutes. Small pupil and nictitating reactions in 10 seconds.

12.24 p.m. Pocket experiment, one minute. Small pupil and nictitating reactions in 13.2 seconds.

12.26 p.m. Pocket experiment, three minutes. Small pupil and slight nictitating reactions in 11 seconds. (Not much different from observation at 12.20.)

12.34 p.m. 0.5 cc. 1: 1,300,000 adrenalin injected. Very large pupil and nictitating reactions in 6.2 seconds.

12.40 p.m. 0.5 cc. 1: 2,600,000 adrenalin injected. Very large pupil and nictitating reactions.

[3] The blood sugar results of this experiment have been already cited in our paper in this Journal, 1917, xliv, 543.

12.41 p.m. 0.5 cc. 1: 2,600,000 adrenalin injected. Same result.
12.46 p.m. 0.5 cc. 1: 2,000,000 adrenalin injected. Very good pupil reaction in 6 seconds. (Nictitating still back.)
12.48 p.m. 0.5 cc. 1: 2,600,000 adrenalin injected. Good pupil reaction in 8.2 seconds.
12.50 p.m. 0.5 cc. 1: 4,000,000 adrenalin injected. Probably slightly larger reaction than that produced by blood collected for two to three minutes in the cava pocket.

Now collected blood specimens from adrenal (short pocket) as follows:
Sample 1, 1.2 grams in 35 seconds (2 grams per minute).
Sample 2, 7.0 grams in four and one-half minutes (1.55 grams per minute).
Sample 3, 9.0 grams in nine minutes (1 gram per minute).
Collected blood from jugular vein and also from abdominal aorta. Left adrenal weighed 0.141 gram and contained 0.15 mgm. epinephrin. Glycogen in liver removed at beginning of experiment, 4.75 per cent.

The eye reactions in this animal indicated that the output of epinephrin could not have been more than 0.00004 mgm. per minute for the animal or 0.000017 mgm. per kilogram of body weight per minute; i.e., not more than one-thirty-fifth of the normal output, as estimated by eye reactions. The rabbit uterus and intestine segment tests showed that the third adrenal specimen contained about 1:65,000,000 epinephrin, corresponding to an output of 0.000015 mgm. per minute for the animal or 0.000006 mgm. per kilogram of body weight per minute; i.e., one-fortieth of the normal as estimated by the segments.

The glycogen content of the liver in these two cats was 4.26 and 4.75 per cent, respectively. In two normal control cats, in which the operative procedure followed in cats 125 and 113 for obtaining a lobe of the liver was imitated under urethane, with ligation of the coeliac and mesenteric arteries, the glycogen content of the liver was 1.95 and 2.55 per cent respectively. In a third normal cat, killed instantaneously without urethane, the content was 4.13 per cent.

Since, as is known, a large proportion of rats survive the excision of both adrenals we made some observations on these animals also. It has been stated by Schwarz (19) that rats do not survive more than a day if both adrenals are removed at one time. He therefore left an interval between the two operations. He is certainly mistaken in this matter for we excised the two adrenals at one time in thirteen rats. Of these eight died in one to fourteen days. The remaining five recovered completely and were sacrificed for the glycogen estimation. The results are given in table 4. In table 5 are given for comparison the glycogen percentages found in five normal rats and in three rats on which a laparotomy had been performed in order to control approxi-

TABLE 4

Adrenalectomized rats

DATE OF GLYCOGEN ESTIMATION	NUMBER OF ANIMAL	DATE OF ADRENALECTOMY	GLYCOGEN PERCENTAGE IN LIVER	REMARKS
1917		*1917*		
November 13	159	October 28	5.01	Ordinary diet*
November 22	162	October 28	2.41	Ordinary diet
December 14	165	December 3	Trace	Unleavened bread and milk; lost weight; accessory adrenal found
December 24	167	December 11	1.40	Ordinary diet (no milk for two days before experiment). Small accessory adrenal found
1918				
January 4	169	December 11	2.99	Unleavened bread, water, no milk; butter for four days

* The ordinary diet for rats consisted of bread, corn, oats and milk daily and a small piece of cabbage once a week.

TABLE 5

Normal Rats

DATE OF GLYCOGEN ESTIMATION	NUMBER OF ANIMAL	GLYCOGEN PERCENTAGE IN LIVER	REMARKS
1917			
November 22	161	2.69	Carrots and sugar for 1 week before experiment in addition to ordinary diet
November 22	163	1.48	Ordinary diet
December 14	166	2.59	Unleavened bread and milk
December 29	168*	2.32	Ordinary diet but no milk for two days before experiment
1918			
January 4	170*	3.98	Unleavened bread, water but no milk (butter four days prior to experiment)
January 25	171*	4.45	Only unleavened bread and water for two weeks before experiment
January 25	172	5.19	Only unleavened bread and water for two weeks before experiment

* To control any general effects of the operation in the adrenalectomized rats, a laparotomy was performed on these three normal rats on December 19, 1917. Adrenalectomy was not performed but otherwise the operation was similar.

mately the effects, as regards trauma, anesthesia, etc., of the operation on the adrenalectomized rats but without removal of the adrenals. No essential difference is shown in the two tables.

Schwarz has stated that the livers of adrenalectomized rats after feeding with dextrose or cane sugar contained considerable quantities of glycogen. His protocols show no definite deficiency as compared with normal rats. On the other hand, he asserts that when carbohydrate is supplied in the form of starch as in feeding "Semmeln," the livers of the adrenalectomized rats are practically free from glycogen, while normal rats with the same diet show a good content. Although the fact that with sugar feeding the adrenalectomized animals form and store considerable quantities of glycogen is sufficient to exclude the idea that any essential change in the process of glycogenesis is caused by the removal of the adrenals, we made some experiments in order to control Schwarz's observations. Two adrenalectomized rats were fed from the time of the operation with a diet certainly free from added sugar, the "matzo" (matzoh), or unleavened bread used during the Jewish Passover. Of these rats, one had a glycogen content of 2.99 per cent. The control rat (170 in table 5) had a content of 3.98 per cent. The other adrenalectomized rat had only a trace of glycogen in the liver but the animal was losing in weight, had been apathetic for some days and was sacrificed because it was feared it was going to die. The control normal rat (166, table 5) had 2.59 per cent. Two other control rats (171 and 172, table 5), were fed solely on unleavened bread and water for two weeks. The glycogen contents were 4.45 per cent and 5.19 per cent respectively.

Kahn and Starkenstein (11) made a few experiments to test the results of Schwarz. They likewise state that rats do not survive when both adrenals are removed at one sitting. Indeed, according to them, if a shorter interval than three to four weeks is left between the first and second operations, death ensues within two days after the second operation. As we have already pointed out, this conclusion is certainly erroneous. A quite considerable proportion of rats survive for a much longer time after simultaneous removal of both adrenals. Kahn and Starkenstein state that adrenalectomized rats fed on a diet of milk, "Semmeln" and some oats, do not store glycogen in the liver except in traces. The number of experiments performed by them was very small and the few protocols given in their paper to not support this conclusion. In the three adrenalectomized animals in which glycogen determinations were made, the glycogen content in one was

2.38 per cent. In another, piqûre had been done before the liver was obtained for the glycogen determination and if a hyperglycemia had been produced the glycogen content before piqûre would doubtless have been considerably higher than that actually found. They did not make any estimations of blood sugar. The interval between the last adrenal operation and the glycogen determination was in general too short for the post-operative depletion of the glycogen store to be certainly made good, especially in view of the fact that they did not purposely feed sugar to the animals. We have had positive results even on a diet containing practically no sugar. That adrenalectomized rats will sometimes show only a trace of glycogen in the liver is true enough, but this is also the case with normal rats. It is necessary before comparing so-called control animals with the operated animals to know that the consequences of the operation as such on the glycogen store have been entirely eliminated. And this can never be assumed with absolute certainty in any particular animal, especially when only a few days have elapsed since the operation. Schwarz anesthetized the rats in order to administer sugar, etc., by the stomach tube and as he fed them in this way on three successive days the effects of the anesthesia may not have been entirely negligible.

SUMMARY

1. In rabbits which have survived the removal of both adrenals and have recovered from the operation, and whose livers are well filled with glycogen, piqûre causes decided hyperglycemia just as in normal rabbits. The hypothesis that piqûre hyperglycemia is caused in the same way as the hyperglycemia produced by injecting adrenalin, by an increased liberation of epinephrin from the adrenals into the blood, must therefore be abandoned. We have previously shown (in cats) that the hyperglycemia associated with asphyxia and with ether anesthesia is likewise not dependent upon the secretion of epinephrin. In the present research we have had many opportunities to observe that hyperglycemia is caused by asphyxia in adrenalectomized rabbits.

2. The results of previous observers who have failed to obtain piqûre hyperglycemia in rabbits after extirpation of both adrenals are due to the fact that they have performed the piqûre immediately after the adrenalectomy, or if an interval has been allowed it has been too short to permit complete recovery from the adrenal operation and the liver has been insufficiently stored with glycogen. Even when a consider-

able interval has elapsed after the adrenal operation, the state of nutrition of the animal or the diet has sometimes been unfavorable for glycogen accumulation and therefore a positive result could not be expected.

3. There is no real evidence that piqûre increases the rate of liberation of epinephrin from the adrenals.

4. It is pointed out that the reactions of denervated vascular regions and of the heart, isolated from extrinsic nervous influence by section of the vagi and excision of the stellate ganglia, which have been interpreted as showing that the rate of liberation of epinephrin is increased by stimulation of afferent nerves and by asphyxia, have a different significance.

5. The formation and storing of glycogen in the liver is not affected by removal of both adrenals in rabbits, or by removal of one adrenal and section of the nerves of the other in cats with consequent abolition or marked reduction in the rate of liberation of epinephrin. In rats, also, extirpation of the adrenals produces no essential change in the capacity of the liver to form and store glycogen.

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NORMAL MECHANISM FOR THE CONTROL OF OXIDATION IN THE BODY

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Lavoisier, shortly after his discovery that oxygen supported combustion, showed that the ingestion of food increased oxidation in the body. Rubner found that of the foodstuffs, meat ingestion increased oxidation most, fat next and sugar least. Several theories have been advanced in attempts to explain how food increases oxidation in the body. Von Mering and Zuntz (1) believed that the increased oxidation following the ingestion of food was due to the increased activity of the intestinal tract. Voit (2), Rubner, Johansson (3) and Benedict (4) have shown that this explanation is not the correct one. Voit believed that the presence of increased quantities of food materials augmented the inherent power of the cells to metabolize. Rubner, on the contrary, contended that the fundamental metabolism of the cell was not effected by the ingestion of food but that the increased heat production which follows the taking of food was due to heat developed from intermediary reactions and oxidations which were in no way involved in the life processes of the cells. Benedict (5) claims that the ingestion of carbohydrates increases oxidation by the formation of acid intermediary products which stimulate metabolism. Lusk (6) holds that the stimulating effect of protein to increased heat production is due to the influx of amino acids which cause an increase in metabolism by their mass action on the protoplasm of the cells.

We (7) have shown that when catalase is increased by the stimulation of the different glands, particularly the liver, to an increased output of this enzyme, there results an increase in oxidation in the body and that when there is a decrease in catalase, a decrease in oxidation follows. The object of the present investigation was to determine if the ingestion of food increases the catalase of the blood and hence of the tissues parallel with the increase in heat production, and if so, how this is brought about and whether protein is more effective in this re-

spect than fat or carbohydrate. Dogs, rabbits and cats were used in the investigation. The form in which the foodstuffs were introduced and the method of introduction will be described later in the paper along with the description of the experiments. Previous to the introduction of the food material at least two determinations were made o the catalase of the blood for the normal. After the introduction of the food materials determinations of the catalase were also made at fixed intervals. The determinations were made by adding 0.5 cc. of blood to a known amount of hydrogen peroxide in a bottle at 22°C. and as the oxygen gas was liberated, it was conducted through a rubber tube to an inverted burette previously filled with water. On account of the low catalase content of the blood of the dogs, 50 cc. of hydrogen peroxide were used while 250 cc. were used for the cats and rabbits. After the volume of gas collected as described in ten minutes had been reduced to standard atmospheric pressure the resulting volume was taken as a measure of the amount of catalase in the 0.5 cc. of blood. The material was shaken in a shaking machine at a fixed rate of one hundred and eighty double shakes per minute during the determinations.

The first part of this paper is concerned with showing that the introduction of the foodstuffs increases the catalase of the blood and hence of the tissues, and that this increase is brought about by the stimulation of the glands, particularly the liver, to an increased output of this enzyme. The fat used was 200 grams of olive oil emulsified by shaking with 100 cc. of a 1 per cent sodium carbonate solution; the sugar, 400 grams of dextose dissolved in 500 cc. of water, and the meat was in the form of a peptic digest. The digest was made by adding 800 grams of ground lean beef, previously freed as much as possible from fat and connective tissue, to 500 cc. of 0.5 per cent hydrochloric acid in which had been dissolved 100 grams of a commercial preparation of pepsin. The mixture was permitted to stand in a thermostat at 40°C. for twenty-four hours. After etherizing a dog that had gone without food for twenty hours, the abdominal wall was opened and the digest of 800 grams of lean meat was introduced into the stomach and intestines in about equal quantities. The acid of the digest was almost neutralized, previous to introduction into the animal, by the addition of sodium carbonate. The material was introduced under pressure through a piece of rubber tubing and a hypodermic needle which was inserted through the wall of the stomach and intestine respectively. In a similar manner the emulsified olive oil and sugar solution were introduced into other dogs. Determinations were made of the catalase of 0.5 cc. of

the samples of blood taken from the portal vein, liver and external jugular vein before as well as at fixed intervals after the introduction of the materials. The blood was taken from the portal and jugular veins by means of a hypodermic needle attached to a 1 cc. pipette. The blood of the liver was collected from a superficial incision made in this organ. Blood thus collected was examined under the microscope and found to be free from liver cells. Bile taken from the gall bladder and tested for catalase was found to contain none. Hence any variation in the catalase content of the blood of the liver from that found elsewhere in the body could not be due either to liver tissue or bile.

In figure 1 curves *A*, *B* and *C* were constructed from data obtained from a dog previous to and after the introduction of the 200 grams of olive oil. Curve *A* was constructed from data obtained from determinations of the catalase in the blood of the liver; curve *B* in the blood from the portal vein and curve *C* in the blood from the external jugular. The figures (0 to 120) along the abscissa indicate time in minutes while the figures along the ordinate (0 to 130) indicate amounts of catalase measured in cubic centimeters of oxygen liberated from hydrogen peroxide in ten minutes by 0.5 cc. of blood. It may be seen that 0.5 cc. of the samples of blood taken from the liver previous to the introduction of the olive oil liberated 80 and 78 cc. of oxygen respectively from hydrogen peroxide; that the blood from the portal vein liberated 75 and 75 cc. and that from the jugular 71 and 72 cc. Fifteen minutes after the introduction of the olive oil the blood from the liver liberated 101 cc. of oxygen, that from the portal vein 80 cc. and that from the jugular 75 cc.; fifteen minutes later, the blood from the liver liberated 105 cc. of oxygen, the portal blood 91 cc. and the blood from the jugular 76 cc.; after forty-five minutes the blood from the liver liberated 111 cc., that of the portal vein liberated 92 cc. and that of the jugular 82 cc.; and after sixty minutes the blood from the liver liberated 116 cc., that of the portal vein 94 cc. and that of the jugular 86 cc.; and after seventy-five minutes the blood of the liver liberated 125 cc.

From these data it may be seen that previous to the introduction of the olive oil the blood from the liver was richer in catalase than that of the portal vein and that the blood from the portal vein, in turn, contained more catalase than the blood from the jugular. It may also be seen that after the introduction of the olive oil the catalase of the blood from the liver increased more rapidly than did that of the portal vein, and the catalase of the blood of the portal vein more rapidly than

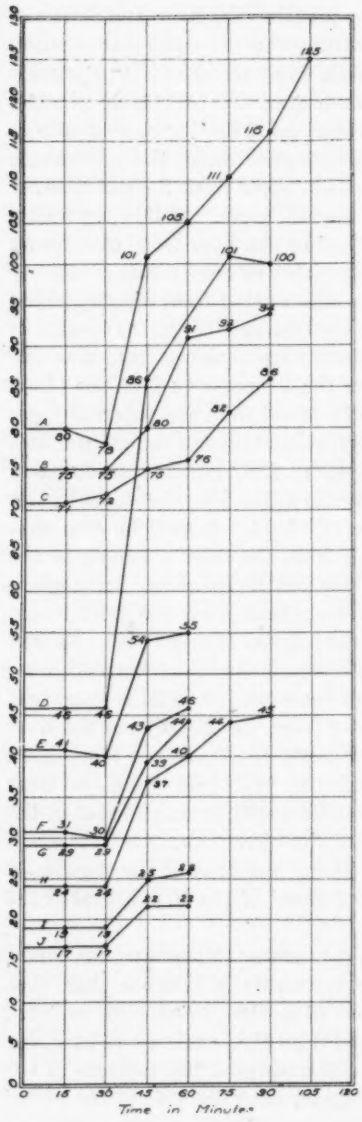

Fig. 1. Curves showing the effect of the introduction of olive oil, meat digest, peptone solution and dextrose into the alimentary tract on the catalase content of the blood; *A*, *B*, *C* the effect of the introduction of olive oil, *D* of meat digest, *E*, *F* and *G* of peptone, *H*, *I* and *J* of dextrose. The figures (0–120) along the abscissa indicate time in minutes; the figures (0–130) along the ordinate indicate amounts of catalase measured in cubic centimeters of oxygen liberated from hydrogen peroxide in ten minutes by 0.5 cc. of blood.

that of the jugular. The fact as shown in curve *B* that immediately after the introduction of the olive oil the catalase of the blood of the portal vein increased more rapidly than that of the jugular, is taken to mean that the gastric and intestinal glands as well as the liver were stimulated to an increased output of catalase by the olive oil. The preceding experiment was repeated on several other dogs and in general the same results were obtained except when smaller amounts of olive oil were used or for some unknown reason, the liver, gastric and intestinal glands were not so strongly stimulated, there was scarcely any change produced in the catalase of the blood of a systemic vein, such as the jugular, although the catalase of the blood of the liver and of the portal vein was always increased. We have found that the catalase of the blood of the systemic veins in general contain between 5 and 20 per cent less catalase than the blood taken directly from the liver or from one of the hepatic veins. This observation is interpreted to mean that while catalase is being given off by the liver, the gastric and intestinal glands, it is being used or destroyed by the tissues. In addition to stimulating the liver and the gastric and intestinal glands, it is probable that other glands, such as the pancreas and spleen, were also stimulated to an increased output of catalase by the olive oil just as we had found to be the case with alcohol.

The same method of procedure was used in studying the effect of the gastric digest, the dextrose and also a peptone solution, as was used in studying the effect of the emulsified olive oil. The peptone solution was made by dissolving 100 grams of peptone in 250 cc. of water. This solution was made acid to the extent of 0.3 per cent by the addition of hydrochloric acid. The results for the gastric digest are given in curve *D*, for the peptone in curves *E*, *F* and *G*, and for the dextrose in curves *H*, *I* and *J*. It may be seen that the effect of the gastric digest, the dextrose and the peptone was in general the same as that of the olive oil, namely, a production of an increase in catalase.

The second part of this paper is concerned with determining whether meat in keeping with its greater stimulating effect on heat production is more effective than fat or sugar in stimulating the glands, particularly the liver, to an increased output of catalase. The animal used was a dog. The foodstuffs were 100 grams of olive oil, emulsified by shaking with 50 cc. of a 1 per cent sodium carbonate solution; the sugar, 225 grams of dextrose dissolved in 400 cc. of water, and the meat, 800 grams of lean round steak freed as far as possible from connective tissue and fat. The emulsified olive oil and sugar solution were introduced

by means of a stomach tube and the animal ate the 800 grams of steak which was finely ground. The catalase of 0.5 cc. of samples of blood taken from the external jugular before as well as at fixed intervals after the giving of the foodstuffs was determined according to the method described in the first part of the paper. There was an interval of about two weeks between the administration of each of the three foodstuffs and the same dog was used for all three of them. The effect of the different foodstuffs given in isodynamic quantities on the catalase content of the blood of the dog is shown in figure 2. The figures along the abscissa indicate time in minutes while those along the ordinate indicate percentage increase in catalase. The curve marked *meat* was constructed from data obtained from the dog after feeding the 800 grams of lean steak; the one marked *fat* after giving the 100 grams of olive oil and the one marked *sugar* after giving the 225 grams of dextrose. It will be seen that two hours after eating the meat the catalase of the blood had increased by 60 per cent; after three hours it had increased 81 per cent and after four hours 115 per cent at which time it had reached a maximum and then began to decrease and had returned almost to the normal amount after nine hours. It may be seen also that the increase produced in catalase by the fat reached a maximum of 60 per cent in three hours and had returned almost to normal after four and one-half hours, and that the effect of the sugar reached a maximum of 51 per cent in two and one-half hours. Comparing the results for the three foodstuffs it will be seen that meat ingestion produced the greatest increase in catalase, fat next and sugar least. This experiment was repeated on the same dog three different times and in general the same results were obtained. The foodstuffs were also fed in isodynamic quantities to several other dogs but none of these dogs gave as good result as those described above. No trouble was experienced in inducing these dogs to eat meat but they would vomit almost invariably after the introduction of either the olive oil or the sugar. In some instances we found, when using a dog whose blood catalase was high, that the feeding even of meat produced very little or no change in the catalase of the blood of the jugular. Upon etherizing and opening the abdominal wall of such a dog, however, and testing the blood taken either directly from the liver or from one of the hepatic veins, we always found the catalase of the blood of the liver to be higher by 50 to 75 per cent than that in the jugular vein, whereas normally the catalase of the blood of the liver is only about 15 per cent higher than that of the jugular. Hence the ingestion of the meat was

stimulating the liver to an increased output of catalase although the blood of the jugular did not indicate it. That was probably due to the rapid destruction of the catalase in the tissues and to the great dilution

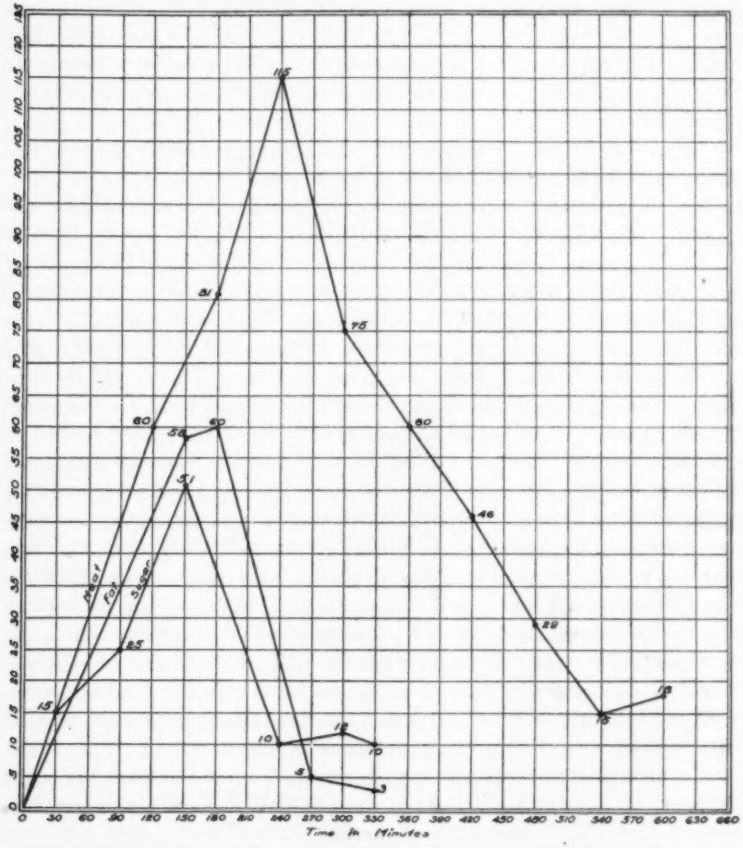

Fig. 2. Curves showing the percentage increase produced in the catalase of the blood by the ingestion of meat, fat and sugar in isodynamic quantities. The figures (0–660) along the abscissa indicate time in minutes; the figures (0–125) along the ordinate indicate percentage increase in catalase.

by the blood. By taking the blood directly from the liver or from a hepatic vein, we have always found a great increase produced in catalase after the ingestion of food.

The last part of this paper is concerned with determining whether the output of catalase from the liver cells continues after the extirpation of the liver, as is the case with the conversion of glycogen into dextrose or the formation of urea from ammonium salts. The animals used were cats and rabbits. After killing the animals the livers were removed and hashed in a hashing machine. The catalase in one gram of the hashed liver was determined according to the method already described.

Curve *A* in figure 3 was constructed from data obtained from determinations of the catalase in one gram of the liver of a cat which was hashed in a hashing machine immediately after its removal from the animal. This hashed material was placed in a glass vessel and covered. A determination of the catalase of one gram of this material was made immediately. After fifteen minutes another determination was made. Similarly, determinations were made after thirty, forty-five, sixty, seventy-five, ninety and one hundred and five minutes. The results of the determinations are given in curve *A*. It will be seen that immediately after the removal and hashing of the liver one gram of the material liberated 820 cc. of oxygen from 250 cc. of hydrogen peroxide. After the material had stood for thirty minutes, one gram liberated 940 cc., after standing one hour, 1060 cc., and after one and one-half hours 1100 cc. From these data it may be seen that the catalase gradually increased in the hashed liver on standing and after two hours it had increased about 34 per cent. This material was kept for about twenty-four hours and it was found that there was no further increase in the catalase content. Curve *B* was constructed from data obtained from a cat in a manner similar to that for curve *A*. Curves *C* and *D* were similarly constructed except the livers of rabbits were used instead of the livers of cats. Similar experiments were carried out on the livers of other rabbits and cats except only a piece of the liver was hashed immediately upon removal from the animal, the other parts of the liver being ground at intervals of thirty minutes immediately before the determinations of the catalase were made. It was found that the catalase increased more rapidly in this material than it did in the livers which were ground immediately upon removal from the animals.

We have shown in this paper that when dextrose reaches the liver after absorption from the stomach and intestines, it stimulates this gland to an increased output of catalase. Claude Bernard (1857) showed that when the liver was removed from an animal the stored

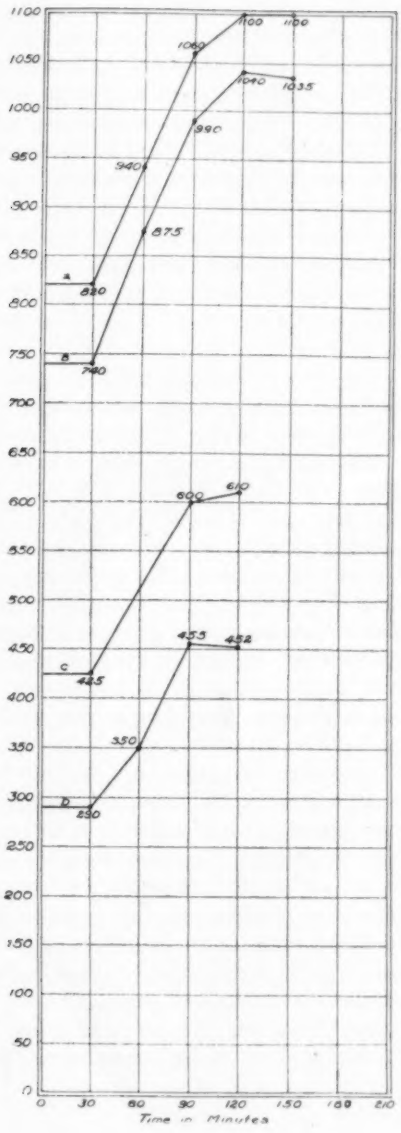

Fig. 3. Curves showing the increase in the catalase of extirpated livers on standing. The figures (0–210) along the abscissa indicate time in minutes; the figures (0–1100) along the ordinate indicate amounts of catalase measured in cubic centimeters of oxygen liberated from hydrogen peroxide in ten minutes by 0.5 cc. of blood.

glycogen was converted very rapidly into dextrose. The explanation that suggests itself for the increase in the catalase of the liver after its removal from the body of the animal is the stimulation of the cells to an increased output of this enzyme by the dextrose arising from the glycogen. This observation would also seem to offer an explanation for the fact that the blood taken directly from the liver or from one of the hepatic veins contains normally more catalase than the blood from any other part of the body. The preceding experiments would seem to indicate that the dextrose, which is being formed continually from the stored glycogen, serves not only as a source of energy when carried to the muscles and oxidized, but acts also to stimulate, particularly in the liver, an increased output of catalase which brings about oxidation, in some as yet unknown way.

We have already shown that when oxidation was increased, as for example, by increasing the amount of work, by thyroid feeding, by fighting and during the excitement stage of ether anaesthesia, there was an accompanying increase in catalase, due to the stimulation of the liver to an increased output of this enzyme, and that when oxidation was decreased or rendered defective, as for example, by decreasing the amount of work, by starvation, by phosphorus poisoning, by extirpation of the pancreas, thus producing pancreatic diabetes with resulting defective oxidation, in deep ether anaesthesia and in "shock," there was an accompanying decrease in catalase. In the present paper it is shown that the end products of digestion stimulate the liver, gastric and intestinal glands to an increased output of catalase parallel with the increase produced in oxidation, and in a previous paper it was shown that during starvation there was a decrease in catalase accompanying the decrease in oxidation. We had also found that a great increase was produced in the output of catalase from the liver by stimulating the splanchnic nerves distributed to this organ. Hence it may be assumed that the output of catalase from the different glands, particularly the liver, is normally controlled by nervous as well as chemical stimuli. In combat, for example, it is probable that the increase in catalase with resulting increase in oxidation is brought about by nervous stimuli reaching the liver over the splanchnics, between meals the output of catalase is controlled principally by the dextrose constantly being formed from the glycogen, during and immediately following meals by the end products of digestion absorbed from the stomach and intestines.

SUMMARY

Ingestion of the foodstuffs increases the catalase of the blood and hence of the tissues parallel with the increase in heat production. The increase in catalase is due mainly to the stimulating effect of the absorbed foodstuffs on the liver. The ingestion of protein in keeping with its greater stimulating effect on heat production produces a greater increase in catalase than fat or carbohydrate. After the removal of the liver from the body of an animal, the liver cells continue to liberate catalase for about two hours, due presumably to the stimulating effect of the dextrose formed from the glycogen.

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